A STUDY ON BACTERIAL AND FUNGAL INFECTIONS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment of the regulations
for the award of the degree of

M.D. (MICROBIOLOGY)
BRANCH – IV

MADRAS MEDICAL COLLEGE,

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON BACTERIAL AND FUNGAL INFECTIONS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR. V.SHINEY JOHN** during the period of her Post graduate study from April 2010 to April 2013 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2013.

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I declare that the dissertation entitled **"A STUDY ON BACTERIAL AND FUNGAL INFECTIONS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL"** *submitted by me for the degree of M.D. is the record work carried out by me during the period of* ***September 2011 to October 2012*** *under the guidance of* ***Prof. Dr. S. VASANTHI, M.D.,*** *Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D.Microbiology (Branch IV) examination to be held in April 2013.*

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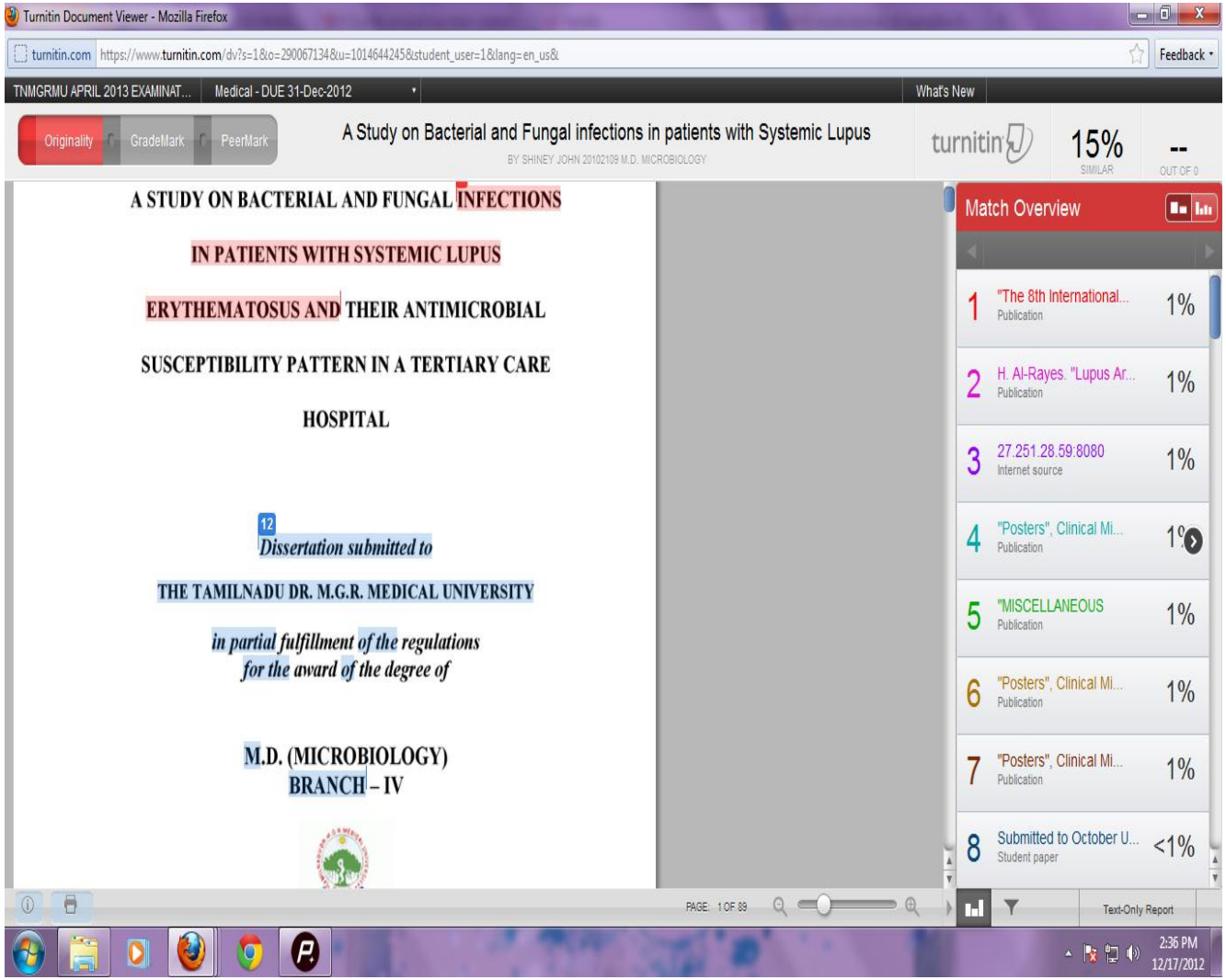
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CONTENTS

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an autoimmune disorder in which multiple systems are affected. In SLE, there is production of autoantibodies, which are directed against nuclear and cytoplasmic constituents of the cell.It is a chronic disease which manifest by periods of alternating exacerbations and remissions. SLE causes injury mainly to skin, joints, and kidney. In certain population the prevalence of SLE may be high. It is a disease which predominantly affects women in their peak reproductive period and is an important cause of morbidity and mortality in that age group of females. Many environmental, genetic and hormonal factors have been associated with the pathogenesis of SLE.

SLE may present with variable symptoms which may range from skin and joint involvement to major organ involvement. The progression of the disease is not predictable. With proper therapy the disease runs a course of flare-ups and remissions which may extent a period of years or sometimes even decades.Corticosteroids and other immunosuppressants are used for treating disease exacerbations. Sometimes the course of SLE may be benign and without taking any medications the patients may survive for years. With recent advances in diagnostic and therapeutic approaches there is significant improvement in the outcome of SLE.

In an individual who is genetically predisposed infections play a key role in the manifestation of the disease. Disease activity and infection are important causes of morbidity and mortality in patients with SLE. It is suggested that infections may also play a main part in exacerbation of disease activity in SLE. Several studies identify active

lupus as a risk factor for infection. SLE patients are inherently at risk for developing infections due to various immunological dysfunctions. Immunosuppressants which are used in the treatment of SLE further increases the susceptibility to infections.

The percentage of SLE patients who develop infections range from 33% to 50%. Infections can develop or supervene during the course of lupus. The risk factors for infection in SLE have been identified in several studies and include active lupus, renal lupus activity, renal insufficiency, proteinuria, low albumin, corticosteroid use and immunosuppressive drug use.

A widerange of infections have been reported in SLE. Bacterial infections are common accounting for 80% of infections. Fungal, viral and parasitic infections have also been recorded in SLE patients. The most common sites of infection in many studies are urinary tract and respiratory tract.

Recognition of infections is important as they may mimic SLE flares leading to a delay in diagnosis or inappropriate increase in doses of immunosuppressants with catastrophic consequences. The published articles on infections in SLE patients from South India are limited. Hence the present study will give an insight into the infections in SLE patients and also the association of infections with steroid intake.

AIMS AND OBJECTIVES

1) To isolate and identify the various bacterial and fungal pathogens associated with infections in patients with SLE.
2) To study the antimicrobial susceptibility pattern of the isolates.
3) To correlate the association of steroid therapy with occurrence of infections in patients with SLE.
4) To study the immunological profile in SLE patients.

REVIEW OF LITERATURE

SLE is the prototype of a systemic autoimmune disorder in which there is production of autoantibodies to constituents of the cell nucleus. SLE affects almost all organs of the body. Thus it is a complex disease and the patients exhibit variable presentations, course and prognosis, remissions and exacerbations which during the course of SLE.

HISTORICAL BACKGROUND

During the middle ages the word LUPUS (Latin for "wolf") was used to denote the skin lesions that were suggestive of a wolf's bite. Lupus was an ancient Roman family name and there was a St. Lupus who lived in France in 600 A.D[13].The word Lupus is attributed to the physician Rogerias Frugardi of 13th century. The term "Lupuserythemateaux" was coined in Paris by the medical student Pierre L.Cazenave in 1851.The visceral involvement in SLE was first recognized by Moriz Kaposi in 1872[20].

This is followed by the following milestones like the discovery of LE cell phenomenon by Malcom M. Hargraves, indirect immunofluorescence microscopy to detect autoantibodies by George Friou *et al* in 1957 and anti DNA antibodies detection in the sera of SLE patients by Tan *et al* in 1966[43] . In 1969 Koffler *et al* in New York found that the antibodies to native double stranded DNA is more specific for SLE. A cytoplasmic ribonucleoprotein antigen designated Sm was detected in SLE serum by Tan and Kunkel in 1966 in New York[43]. Criteria for classification of SLE have been established and revised by the American college of Rheumatology in 1982[5, 32].

The history of lupus is divided into three periods:

a) Classical period: In 1846 the Viennese physician Von Hebra compared the malar rash to butterfly. He was also the one to use the term lupus erythematosus.
b) Neoclassical period: This was marked by the recognition of lupus as a distinct disease with systemic manifestations.
c) Modern period: This period was initiated by the discovery of the LE cell in 1948, followed by the discovery of autoantibodies, pathophysiology and improved therapy.

EPIDEMIOLOGY AND CLASSIFICATION

EPIDEMIOLOGY

SLE was considered a rare disease till 1950s. As of 1936 five cases of SLE were found among 7,500 autopsies at the John Hopkins Hospital. There had been an increase in the number of cases since then due to increased use of the LE cell test and other diagnostic methods.

The reported prevalence of SLE in India ranges from 14 to 60 per 100,000[55]. In United States the prevalence of SLE is approximately 51 per 100,000[2]. As a result of early diagnosis of SLE the incidence has increased three times in the last 40 years[2].The female to male ratio is estimated to be around 9:1. Race also determines the predisposition to develop SLE, African-Americans and Hispanics are frequently affected than whites. The disease morbidity is also high in them[13]. In a study conducted in Saudis the sex ratio is 4.4: 1 with an average age of 35years at onset of disease[13]. In India the median age of onset of SLE is estimated to be 24.5 years and the female to male ratio

(F:M) is 11:1[56]. 65% of patients with SLE have onset of disease between the ages of 16 and 55, 20% have onset before the age of 16 and in remaining 15% after the age of 55[20].

The features of male SLE differ in various ways from female SLE like less occurrence of photosensitivity, more occurrence of serositis, diagnosis at an older age and an increased one year mortality rate. In elderly patients with SLE, the disease is milder and they have a lower occurrence of clinical features like malar rash, photosensitivity, alopecia, renal involvement, neuropsychiatric manifestations but they have a higher occurrence of serositis and lung involvement[20].

The survival period for SLE patients has improved considerably; but when North American and European repots were compared the survival rate was low among SLE patients in India[52].

CLASSIFICATION CRITERIA

In 1971 the classification criteria were developed. The criteria were revised in 1982 and again in 1997[20]. The American college of Rheumatology has established the following criteria for SLE classification. To be classified as lupus, a patient must satisfy four out of eleven criteria. These criteria are valuable in clinical and laboratory investigation of this disease since they easily differentiate patients with Lupus from those with other connective tissue diseases.

1997 AMERICAN COLLEGE OF RHEUMATOLOGY CLASSIFICATION CRITERIA FOR SYSTEMIC LUPUS ERYTHEMATOSUS

1) Malar rash(butterfly rash)
2) Discoid rash
3) Photosensitivity
4) Oral ulcers
5) Arthritis- Nonerosive arthritis involving two or more peripheral joints, with tenderness, swelling, or effusion
6) Serositis- Pleuritis or Pericarditis
7) Renal disorder- Proteinuria >0.5g/dl
8) Neurologic disorder- Seizures or psychosis without other causes
9) Hematologic disorder- Hemolytic anemia or leukopenia or thrombocytopenia in the absence of offending drugs
10) Immunologic disorder- Anti- ds DNA, anti-Sm, and /or anti-phospholipid
11) Antinuclear antibodies- A significant titre by immunofluorescence or other assay in absence of drugs known to induce ANAs.

A person shall be said to have SLE if any four or more of the eleven criteria are satisfied, serially or simultaneously during any interval of observation.

PATHOGENESIS AND ETIOLOGY

Several genetic, environmental and immunological factors have been found to be associated with the pathogenesis of SLE.

a) GENETIC FACTORS

There are strong evidences to suggest that SLE is a genetic disease. Both MHC and non-MHC genes contribute to the risk of developing SLE. This is supported by the fact that the disease shows aggregation in family, higher rate of concordance in twins and finding that prevalence and severity of SLE differ between ethnic groups[42].

Genes associated with SLE

1) HLA Class II alleles (HLA – DR and HLA –DQ)
2) Complement gene defects causing deficiencies of:
    - C1q or C1r / C1s
    - C2
    - C4 (C4A and C4B), C4A
3) Mannan-binding protein gene deficiencies
4) Fcγ receptor alleles with differences in function:
    - Fcγ RIIA
    - Fcγ RIII
5) Cytokine gene alleles causing differences in expression:
    - TNF – α, IL – 10, IL – 6

b) IMMUNOLOGICAL FACTORS

Dendritic cells play a role in initiation and later amplification of autoimmune response in SLE by abnormally presenting self antigens which are derived from damaged and apoptotic cells to T cells. The central players of autoimmunity in SLE are B cells. They mediate tissue injury by producing high levels of autoantibodies. Also, since B cells can also act as antigen presenting cells, they internalize the soluble antigens and present them to T cells. There is decreased threshold and increased inflammatory capacity of T cells. So there is enhancement of T-cell effector function[42].

c) ENVIRONMENTAL FACTORS

There are many evidences to support the association of estrogens with SLE. Estrogens increase the risk of SLE or cause exacerbation of disease activity. Several studies have documented increased risk of SLE in smokers. EBV specific immune responses have been implicated in SLE pathogenesis.

Ultra violet exposure may act as a triggering factor for SLE disease flares. It has been found that ultraviolet light induces apoptosis of cells and the DNA is altered so that it becomes immunogenic[43].

AUTOANTIBODIES IN SLE

The humoral immune response protects our body from pathogenic organisms by forming antibodies that either destroy or inactivate microbes and also their toxins. To do this function of protecting our body our immune system produces antibodies to a varied

array of foreign antigens, but it must do without generation of pathogenic antibodies to self.

The characteristic feature of SLE is the production of high affinity antibodies that are able to bind self- antigens. It has been found that antibodies like anti-Sm/RNP, ANA are markers for disease while other antibodies are thought to play a significant role in pathogenesis of SLE. The pathogenicity of autoantibodies is determined by the heavy chain isotype.

Even in healthy individuals a degree of autoimmunity or self-reactivity occurs. In autoimmune diseases the autoantibodies have high affinity for self antigens through somatic mutation. The other features in SLE include hyper responsiveness of B cells, polyclonal hypergammaglobulinemia, and there is also high antibody titres to viral antigens which are encountered commonly.

The production of autoantibodies is an important feature of SLE. Autoantibodies in SLE are of IgG (IgG1 & IgG3) and to a lesser extent, IgM and IgA isotypes[89].There is a polyclonal immune response to each autoantigen.

ANAs cannot penetrate intact cell, but if cell nuclei are exposed can bind with them. LE bodies or hematoxylin bodies are produced when the nuclei of damaged cells react with ANAs, losing their chromatin pattern and becoming homogenous. LE cells are easily seen when blood is agitated in vitro, which is any phagocytic leukocyte engulfing the denatured nucleus of injured cell. In the past LE cell demonstration was used as a test for SLE, but it is now largely of historic interest [89].

SPECIFIC AUTOANTIBODIES

Autoantibody in SLE are referred to as antinuclear antibody(ANA)

1) ANTIBODY TO DNA AND DNA-BINDING PROTEINS

a) Anti-DNA

It is found that three forms of DNA are antigenic in SLE: single stranded DNA, double stranded DNA and left handed (Z)DNA. Anti ds-DNA antibodies are more specific for SLE and found in 60% of patients with SLE and they reflect disease activity. There has been evidence for the role of DNA-anti DNA immune complexes in the pathogenesis of lupus nephritis[89].

b) Antihistone and antinucleosome

The intact DNA in eukaryotes forms a double stranded helix due to pairing between complementary bases on each strand and is compacted into chromatin through its interaction with histones and other proteins. Nucleosome is a subunit of chromatin and it comprises about 200 base pairs of DNA twisted around an octamer of histone (two molecules each of H2A,H2B,H3 and H4). Histone H1 binds on the surface and link adjacent nucleosomes and stabilizes the nucleosome.

In 50-70% of SLE patients with antihistone antibodies they target the H1 and H2B proteins, followed by H2A, H3 and H4 usually in association with anti-ds DNA antibodies. In drug induced lupus these antibodies occur in association with anti ss-DNA antibodies. Procainamide and Quinidine induce IgG antibody reactive with (H2A-

H2B)DNA complex. Hydralazine and Chlorpromazine induce IgM antibody that bind to H1, H3-H4, H2A-H2B and ss-DNA[89] .

c) Anti-Ku(nuclear factor IV)

Ku is a heterodimer made of two proteins of 70KDa and 86KDa and has a role in DNA repair. Both diffuse nuclear and nucleolar immunofluorescence staining produced by Anti-Ku antibodies. They are found in scleroderma-myositis overlap, scleroderma and SLE.

d) Anti-proliferating cell nuclear antigen(PCNA)

PCNA is a 36KDa protein which is an auxiliary protein of DNA polymerase delta and it produces positive IF staining only in dividing cells[89].

2) ANTIBODIES TO RNA AND TO RIBONUCLEOPROTEINS

a) Anti-RNA

Autoantibodies to Single stranded (SS) and double stranded (ds)-RNA have been documented in 60% of patients with SLE[89].

b) Anti-Sm, Anti RNP

Anti-Sm antibodies are found only in SLE patients, but anti- RNP antibodies occur in other autoimmune disorders also.

c) Anti-Ro (SS-A) and anti-La (SS-B)

La is a nuclear phosphoprotein that binds to precursor transfer(t) RNAs and 5s rRNA and to small RNAs encoded by viruses[89]. Two types of Ro proteins are thoroughly characterized Ro60 and Ro52 and are present in both cytoplasmic and nuclear

compartments. Antibody to Ro 60 (SS-A) is found in 25-40% of patients with SLE and antibody to La (SS-B) is found in 10-15%[89]. Anti Ro/La antibodies are associated with subacute cutaneous lupus erythematosus, photosensitivity and neonatal lupus erythematosus syndrome. IgG anti-Ro crosses placenta and can bind to conduction system of fetal heart, can alter myosin-actin function and cause heart block. Most of the infants with congenital heart block have mothers with anti-Ro antibodies[20].

d) Antiribosomal antibodies

Antiribosomal antibodies directed against three phosphoproteins (P proteins)- P0, P1, and P2 located on the large (60 S) ribosomal subunit. In SLE the prevalence of anti-Rib-P antibodies is reported to be around 10% to 40%[89].

3) ANTIBODIES TO CELL MEMBRANE COMPONENTS

a) Antibodies to blood cells

SLE patients have autoantibodies directed against red cells, platelets, and lymphocytes.

b) Antineuronal antibodies

IgG and IgM antibodies that bind to the surface of neuroblastoma cells in vitro and other neuronal antigens have been detected in the serum of patients with SLE[89]. The presence of IgG antineuronal antibodies in the CSF correlate best with CNS involvement in SLE.

c) Antibodies to stress or heat shock proteins (hsp)

IgM antibodies to the 73 KDa member of the hsp 70 family and IgG antibodies to hsp 90 families have been identified in a small proportion of patients with SLE but their pathogenic significance remains to be established[89] .

d) Antiphospholipid antibodies (aPL antibodies)

They form a heterogenous group and are directed against anionic phospholipids (PL) or protein-phospholipid complexes. They are called lupus anticoagulants(LACs) because they prolong phospholipid dependent coagulation assays[62]. aPL antibodies occur in 40% -50% of SLE patients. The antibodies against the phospholipid-β2-glycoprotein complex have the ability to bind cardiolipin antigen which is used in serological test for diagnosing syphilis. SLE patients may therefore give false-positive test result for syphilis.

DETECTION OF ANA

1. INDIRECT IMMUNOFLOURESCENCE METHOD

The indirect immunofluorescence ANA test (FANA) provides a sensitive screening method for ANA detection[89]. Test sera at varying dilutions are incubated with substrate cells and bound antibodies are detected by fluorescein conjugated anti-human Ig followed by visualization under a fluorescence microscope. Its reliability depends primarily on the choice of substrate, which may vary from rodent liver or kidney frozen sections to cultured proliferative cell lines, most commonly the human epithelial tumor cell line HEp2.

Because different ANAs have different intracellular targets, clinically useful information may be obtained by observing the fluorescence pattern in the nucleus,

nucleolus or cytoplasm. Nuclear patterns include homogenous, rim and speckled staining. Nucleolar patterns include discrete speckled, grainy speckled and clumpy staining. Normal individuals usually older and female persons or relatives of persons with connective tissue diseases positive FANA results at a frequency of upto 30% although their titer generally remains below 1:320 and the pattern is often homogenous.

2. IMMUNODIFFUSION (ID)

The double immunodiffusion Ouchterlony technique provides a crude method for the detection of autoimmune serum specificity by the comparison of precipitin activity with prototype antisera. Although ID provides a simple method for the detection of specific autoantibodies, its low sensitivity compared with newer assays limit its widespread use[89].

3. COUNTERIMMUNOELECTROPHORESIS (CIE)

The CIA technique modifies the ID method to give greater sensitivity. Acidic antigens such as DNA or RNA are electrophoresed from a cathode well and antibodies from an anode well. A precipitin line forms as specific autoantibodies encounter antigen. Because this method also provides limited sensitivity with a limited repertoire of autoantibody detection, it has largely become supplanted by other methods of ANA detection[89].

4. **ELISA**

ELISA provides a highly sensitive and rapid technique for the detection of ANAs and antibody specificity. Sometimes ELISA tends to produce false positive results and confirmation requires further testing.

5. IMMUNOPRECIPITATION

Radio immunoprecipitation assays provide sensitive and specific means to detect autoantibodies.

6. **IMMUNOBLOTTING**

Western blotting assays use autoimmune serum as probes against membranes containing electrophoretically resolved purified or crude antigens. Bound antibodies are detected by an enzyme linked antihuman IgG antibodies and substrate dependent colour development.

ANTI- DNA ANTIBODY TESTS

1. FARR RADIO IMMUNO ASSAY (RIA)

The Farr RIA, which resembles immunoprecipitation assays involves the binding of autoantibodies to radio labeled ds DNA in solution and this assay is generally considered to be the gold standard for anti ds DNA analysis[89].

2. CRITHIDIA LUCILIAE TEST

Crithidia luciliae test provides an inherently reliable ds DNA substrate. In this assay, the hemoflagellate Crithidia luciliae serves as a substrate for indirect immunofluorescence. Its kinetoplast, a modified giant mitochondrion contains a concentrated focus of stable, circularized ds DNA without contaminating RNA or nucleoproteins providing a sensitive and fluorescent substrate to establish anti ds DNA activity[89].

3. **ELISA**

ELISA kits are now available for detecting anti ds DNA antibodies.

CLINICAL FEATURES

The clinical features of SLE are variable from general manifestations to organ-specific manifestations.

GENERAL MANIFESTATIONS

The general manifestations in SLE patients include malaise, fatigue, fever, weight loss and anorexia.

CUTANEOUS MANIFESTATIONS

Cutaneous manifestations in SLE are classified as Lupus Erythematosus (LE) specific and LE non specific.

a) LE specific lesions

LE specific lesions in SLE can be classified into acute, subacute and chronic lesions. **Acute lesions**: Acute lesions may present as localized or generalized lesions. Butterfly or malar rash is a localized skin lesion in SLE[4].

Subacute Cutaneous Lupus Erythematosus (SCLE): SCLE denotes the cutaneous lesion which is widespread in distribution, photosensitive and non-scarring[4].

Chronic Lupus: Chronic lupus manifest as discoid lesions in the absence of other systemic symptoms and known as discoid lupus. They may also be a disease manifestation of SLE[4].

b) LE non-specific lesions

The non-specific lesions correlate with lupus activity and include alopecia, vesiculobullous lesions, urticarial lesions and vasculitic lesions.

Photosensitivity

Photosensitivity may occur in more than 50% of patients with SLE[4].

MUSCULOSKELETAL MANIFESTATIONS

a) Arthritis

Arthritis is one of the most common manifestations in SLE. The joint involvement may be in the form of arthritis, arthralgia or both and is classically non-erosive and non-deforming involving small joints of the hands, wrists and knees[20].

b) Myositis

Myositis is one of the causes of myalgia in SLE patients. Myositis of proximal muscles is found to occur in 5% to 11% of patients[82].

c) **Osteonecrosis**

Osteonecrosis is common in younger age group and present early in the disease course. Symptomatic osteonecrosis occurs in 5% to 12% of patients with SLE[23].

RENAL MANIFESTATIONS

Renal involvement occurs in about 40% to 70% of SLE patients[20]. It is also a major cause of morbidity and mortality in them. Renal biopsy is done to find the histologic class of Lupus Nephritis (LN).

TABLE I: Histologic classification of Lupus Nephritis

WHO TYPE	
CLASS I	Minimal mesangial lupus nephritis
CLASS II	Mesangial proliferative nephritis
CLASS III	Focal lupus nephritis
CLASS IV	Diffuse lupus nephritis
CLASS V	Membranous lupus nephritis
CLASS VI	Advanced sclerotic lupus nephritis

The above classification is as per the International Society of Nephrology / Renal Pathology Society.

Patients with membranous glomerulopathy have proteinuria, C_3tends to be normal and anti-DNA antibodies are found in low titres. But patients with proliferative nephritis have hypertension, a nephritic urine sediment with proteinuria, low C_3 and high titres of anti-DNA antibodies.

Prognosis varies among the clinical and pathologic forms of lupus nephritis. The factors associated with bad outcomes include African-American race, azotemia, anemia,

APS, failure to respond to initial immunosuppressive therapy and flares with worsening in renal function[9, 60].

NEUROPSYCHIATRIC MANIFESTATIONS

Nervous system involvement is a major cause of morbidity and mortality in SLE. SLE affects the CNS and the peripheral nervous system. The syndromes involving nervous system in SLE are collectively called neuropsychiatric systemic lupus erythematosus (NPSLE) syndromes[3]. Cognitive dysfunction is reported in 80%, psychosis in 8%, generalized and focal seizures in 6% to 51% and peripheral neuropathy in 28% of SLE patients.

CARDIAC MANIFESTATIONS

Pericarditis is the most common manifestation in SLE.

PULMONARY MANIFESTATIONS

The pulmonary manifestations include abnormal pulmonary function, pulmonary hemorrhage, pulmonary embolism, lupus pneumonitis and pulmonary hypertension[4].

LYMPH NODE AND SPLEEN INVOLVEMENT

Lymphadenopathy occurs in 40% of SLE patients usually at disease onset or during disease flares. Splenomegaly occurs in 10% to 45% of patients during active disease[20].

HEMATOLOGIC ABNORMALITIES

a) Anemia

Anemia occurs in about 50% of SLE patients. The causes of anemia in SLE patients include anemia of chronic disease, iron deficiency anemia and autoimmune hemolytic anemia[4].

b) Leukopenia

Leukopenia is common in SLE and usually associated with disease activity. A WBC count of less than 4,500 / μL is reported in 50% of SLE patients, especially patients with active disease. Lymphocytopenia occurs in 20% of SLE patients. Decreased eosinophil and basophil counts are secondary to corticosteroid use in lupus. Leukocytosis can occur in lupus and reflects an infection or the use of corticosteroids in high doses[20].

c) Thrombocytopenia

Mild thrombocytopenia occur in about 25% to 50% of SLE patients[20].

LIVER AND GASTROINTESTINAL TRACT INVOLVEMENT

Gastrointestinal manifestations have been reported in 25% to 40% of patients with SLE and could be due to either lupus of the gastrointestinal tract or the effects of medications[20].

OPHTHALMIC INVOLVEMENT

Retinal vascultitis occurs in a small percentage of SLE patients and is usually associated with active disease[20].

TREATMENT OF SLE

SLE is not a curable disease and it is rare to see complete remissions. The treatment plan is therefore to control severe exacerbations of disease and to develop subsequent maintenance strategies to suppress symptoms and prevent organ damage.

Treatment of mild SLE without major organ involvement

Nonsteroidal anti-inflammatory drugs (NSAIDs), antimalarial (hydroxychloroquine), glucocorticoids and in severe cases immunosuppressive agents (azathioprine, mycophenolatemofetil, methotrexate) are used.

Treatment of moderate to severe SLE

Treatment consists of a period of induction therapy in which intensive immunosuppressive therapy is given to the patient and this is followed by a longer period of maintenance therapy using less intensive immunosuppressants[9].

Corticosteroids

For patients with moderate to severe disease corticosteroids are used either as single or as background therapy in combination with immunosuppressive agents at prednisolone doses ranging from 0.5 - 1.0 mg / kg / day in a single dose followed by

tapering of dosage. In cases of doses more than 0.6 mg / kg / day or in rapidly progressing severe disease bolus therapy is used (1000 mg of methylprednisolone IV daily for three days followed by 0.5 mg / kg / day of prednisolone[20]

Azathioprine

In moderate to severe SLE, Azathioprine is used at doses of 1 - 3 mg / kg / day[20].

MycophenolateMofetil (MMF)

MMF is used to treat manifestations of SLE like proliferative nephritis, skin disease, refractory thrombocytopenia and pulmonary haemorrhage in patients refractory to corticosteroids or cytotoxic agents[20].

Cyclophosphamide

Intermittent pulse cyclophosphamide therapy (IV cyclophosphamide) is effective for moderate to severe proliferative lupus nephritis. The treatment can be given as follows.

(1) Once monthly IV for 6 months followed by two years of quarterly doses,
(2) Cyclophosphamide for 12 weeks followed by azathioprine
(3) Cyclophosphamide for 6 months followed by azathioprine or mycophenolatemofetil[48].

Rituximab

Rituximab is an anti-CD20 chimeric murine / human monoclonal antibody that depletes B cells but not plasma cells – resulting in peripheral blood B cell lymphopenia[20].

Other Drugs used in the treatment of SLE

- Methotrexate
- Cyclosporine
- Intravenous Gamma Globulin

INFECTIONS IN SLE

SLE patients are prone to develop infections due to the underlying immunological dysfunctions. The immunosuppressive agents that are used in treating sever disease manifestations further increases this risk of infections.

SLE mortality from infections

In reviewing mortality from a forty year period, Hellman *et al*. attributed 27% of deaths in SLE patients to infections. In another study by Klemperer *et al*. infection was the cause of death in 40% of SLE patients. In a large multicenter study of 1103 patients with SLE 33% of the deaths were caused by infection, and infection was a contributing cause in 10% of patients[77]. When clinical and demographic features such as age, socioeconomic status and race were considered infection was the leading cause of death in all groups (39%) by Reville *et al*.[74]

A 1993 report from Thailand documented infection as the cause of death in 23 of 77 patients with SLE[13]. In European and Asian series reported in the late 1990s, despite wide ranges in case fatality rates of 4.5% to 24%, the proportion of deaths attributed to infection was similar, ranging from 20.5% to 32.5%[13]. In a case-control study done by Irastorza *et al*. to identify major infections in 83% SLE patients infection was the cause of death in 8 patients[25].

Reveille *et al*.[74], Breban *et al*.[10], Abu Shakra *et al*.[2], Bellomio *et al*.[7]and Mok *et al*.[59]have ranked infections as the first cause of death in patients with SLE whereas infection was ranked as the second cause of death in patients with SLE by Urmanand Rothfield[84], Feinglass *et al*.[19], Wallace *et al*.[87], Halberg *et al*.[27], Hashimoto *et al*.[29] and Michet *et al*. [57].

Rates of infections in SLE

In a Swedish study in 1985, Nivel *et al*[13] found an overall infection rate of 142 per 100 patient years of SLE. The 1974 report of hospitalized SLE patients by Staples *et al*[79] identified an overall culture verified infection rate of 1.22 per 100 hospital days.The most common infection site was the urinary tract. A 1991 review of infections in hospitalized SLE patients found an infection rate of 1.94 per 100 hospital days. In a recent study, serious infections developed in one-third of lupus patients during follow-up[25]. In a study conducted in Korea 38% had at least one episode of infectious disease and the incidence of infectious disease was 4.4/100 patient-years[39]. H-Al-Rayes *et al*. reported infections in 58.79% of SLE patients in their retrospective study[26].

Factors influencing susceptibility of patients to infection

Many investigators agree that treatment with corticosteroids, as well as some manifestations of active SLE itself play important role as risk factors that predispose to infections in SLE patients. Even in the absence of corticosteroid, infections are common in SLE patients. Specific renal measures notably active urinary sediment was a significant predictor of infection. Nephritic syndrome and uremia associated with an increase but not significant incidence of opportunistic and bacterial infections[92].

A strong association was found between blood urea nitrogen and infections suggesting poor renal function are more important than renal inflammation. An increased tendency to develop bacterial and opportunistic infections is documented even among patients with SLE in clinical remission[34].

In a study done in Singapore, an increase in bacterial infections was observed among patients with SLE compared to normal controls, whereas the incidence of fungal and viral infections was similar in controls and patients with SLE[66].

Other abnormalities which account for this susceptibility to infection include reduction in immunoglobulin, defects in chemotaxis, phagocytic activation and delayed hypersensitivity, acquired and inherited deficiency of complement. Activation and consumption of complement and the role of specific complement components has been defined in SLE. C3 deficiency associated with increased risk of recurrent or disseminated bacterial infections. Hereditary deficiencies of complement components also have been described in patients with SLE[71]. Repeated skin and upper airway infections,

Haemophilus influenza septicemia and bacterial meningitis occur due to autosomal recessive defects in c1q, c1r and c2 production and homozygous deficiencies of c2. Patients with decreased complement components C5-9 are prone to infections with encapsulated organisms such as Neisseria species. Patients with SLE who had a serum inhibitor of C5 derived chemotactic activity had increased incidence of infections[30].

Abnormalities of reticuloendothelial function also occur in SLE patients. The circulating immune complexes cause saturation of Fc receptors in liver and spleen thus preventing clearance of opsonized bacteria. This may be the cause of pneumococcal bacteremia and chronic *Salmonella* carrier state seen in some patients with SLE. The incidence of Herpes Zoster is increased in patients with SLE compared with normal individuals which may be due to abnormalities of cellular immunity[61].

Staples *et al*[79] found that the infection rate in hospitalized patients increase from 0.43 to 1.63 per 100 hospital days with increase in steroid dose from zero to >50 mg/day. In the Downstate study five fold increase in frequency of infection was found, ranging from 35 to 179 per 100 patient-years as average prednisone dose increased from zero to greater than 40mg/day.

Risk factors for infection were glomerulonephritis, severe flare, corticosteroids, cyclophosphamide and plasmapheresis[13]. Corticosteroids predispose to infection by affecting host responses to microorganisms which include a decreased inflammatory response, decreased effector cell response in cell mediated immunity, lysis of lymphoid follicles and decreased immunoglobulin synthesis.

Prolonged steroid administration increases risk of infection by causing chronic changes in tissues such as skin atrophy which allow increased access of microorganisms into the circulation. A regimen of alternate day steroid administration is believed to decrease risk of infection[13]. Other immunosuppressive agents like azathioprine and cyclophosphamide are implicated as risk factor for infection in patients with SLE[13].

A French study of cytolytic therapy in rheumatic diseases failed to find an increased risk of infection, with the exception of Herpes virus, which was associated with a 10% to 20% incidence of infection compared with 2% in patients not treated with immunosuppressants. A prospective study in Singapore did not show an association of cytotoxic therapy and infection in patients with SLE[66].

It was found that patients treated with plasmapheresis and cyclophosphamide had more chances of developing infections compared to patients treated with cyclophosphamide alone. Recent clinical trials suggest infections are less frequent in patients treated with Mycophenolatemofetil as compared to Cyclophosphamide[13].

SPECIFIC TYPES OF INFECTIONS

Bacterial infections

Of all the infections occurring in SLE patients, bacterial infections are the commonest accounting for 50%[13]. The most common sites of bacterial infections are similar in SLE patients and in persons without SLE which include urinary tract, respiratory tract and skin. H-Al-Rayes in their retrospective study in SLE patients observed that bacterial infection was predominant (78.6%). This is followed by viral

(28.2%) and fungal (28.2%) infections[26]. The most common organisms isolated are *Staphylococcus aureus, Escherechia coli, Klebsiella spp*. and *Pseudomonas spp*. In hospitalized patients with SLE bacteremia is common [13].

In a recent study the most common infections reported were pneumonia, bacteremia and skin and soft tissue infections. The most frequent isolates in this study were *Escherichia coli, Staphylococcus aureus, Mycobacterium tuberculosis* and *Streptococcus pneumoniae*[25].

The organisms which cause infections in healthy individuals occur with increasing frequency in unusual sites in patients with SLE. Opportunistic infections caused by *Salmonella typhimurium* and *Salmonella enteritidis* have also been reported in patients with SLE. Infection with *Listeria monocytogens* is also documented and is associated with risk factors like renal failure, disease flare, and other immunosuppressants. Tuberculosis (TB) is also common in SLE. In a study done by Fang and Tan, TB was found in 5% of patients with SLE. A recent study reported extrapulmonary and military TB as common in patients with SLE[13].

Skin involvement due to *Atypical Mycobacteria* is rarely reported in patients with SLE. There are also few case reports of unusual bacterial infections like Legionnaire's disease, *Campylobacter* endocarditis, Toxic shock syndrome and *Pseudomonas pseudomallei* meningitis. Infection with *Treponema pallidum* may occur, although false positive serologic tests for syphilis occur in SLE[13].

Viral infections

The most common specific viral infection in patients with SLE is Herpes zoster. The incidence in Western countries ranges from 3% to 21%, which is higher than that observed in general population. In a study conducted in Japanese patients with SLE, 43% incidence has been reported. Herpes simplex associated with esophageal and perianal lesions are also common in patients with SLE. Other viruses associated with infection in SLE patients include *Cytomegalovirus, Human Papilloma Virus* (HPV) and *Parvovirus B19*[13].

Fungal infections

The most common fungal infection is caused by *Candida spp*., mostly presenting with oral thrush. Esophageal candidiasis may co-exist with Herpes simplex. The risk factors for the development of deep fungal infections in SLE patients include corticosteroid and immunosuppressive therapy and most common organisms are *Candida spp*. and *Cryptococcus*. Zygomycosis is associated with thrombotic thrombocytopenic purpura, CNS complications and increased mortality rate. Infections with *Aspergillus spp* have also been documented. There are case reports of fatal *Aspergillus* meningitis and septicemia in patients with SLE. Other fungal infections reported in SLE patients are Coccidioidomycosis, Nocardia infections, disseminated histoplasmosis and maduramycosis. Wei-I Tsai *et al*.[88] in their study on invasive fungal infections have reported the most lethal species in the following order - *Cryptococcus, Aspergillus spp*. and *Candida spp*.

Parasitic infections

Strongyloides stercoralis hyperinfection mimicking an SLE flare may occur. One case each of Visceral leishmaniasis and Paragonimiasis have been reported. Infection due to *Toxoplasma gondii* have also been reported[13].

A high index of suspicion is essential as the symptoms of SLE and infection are often similar. The helpful clues which help to diagnose infections are presence of the following like chills, leukocytosis, increased C Reactive Protein (CRP) levels[80]and absence of SLE involving multiple systems. Hence SLE patients are at high risk for developing infection. So all fevers must be evaluated in SLE patients because the most common causes of fever in them are infections and active lupus. Also the signs of inflammation in these patients may be altered by steroids and NSAIDs which are used for treating disease manifestations. Thus careful use of steroids and other immunosuppressive agents in SLE patients is recommended to limit infections.

MATERIALS AND METHODS

PLACE OF STUDY

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College in association with Department of Rheumatology, and Department of Nephrology, Rajiv Gandhi Government General Hospital, Chennai.

STUDY PERIOD

The study period was for one year from September 2011 to October 2012.

ETHICAL CONSIDERATION

Approval was obtained from the Institutional Ethical Committee before the commencement of the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

STUDY POPULATION

Total of 110 patients fulfilling the 1982 revised American Rheumatism Association criteria for the classification of SLE with clinically suspected infections were included in the study.

CASE DEFINITION

Infection in SLE patients is suspected based on clinical data like fever, lower abdominal pain, frequency / pain during urination, cough with expectoration, loss of weight, and skin lesions.

EXCLUSION CRITERIA

SLE patients with malignancy, transplant recipients, prolonged antibiotic therapy, and HIV were excluded from the study.

DATA COLLECTION

Data collection included age, sex, and duration of SLE, clinical features, laboratory investigations, and steroid/cytotoxic therapy.

STATISTICAL ANALYSIS

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) and graphpad software by a statistician.

SAMPLE COLLECTION, TRANSPORT AND PROCESSING

Under strict aseptic precautions, samples were collected from the patients and transported immediately to the laboratory and sample processing was done.

SAMPLES COLLECTED

1) Urine
2) Sputum
3) Bronchoalveolar lavage (BAL)
4) Ascitic fluid
5) Cerebrospinal Fluid (CSF)
6) Oral scrapings
7) Nail
8) Pus
9) Blood
10) Faeces

COLLECTION OF SAMPLES

Urine

Freshly voided clean catch midstream urine samples were collected in sterile wide mouthed containers.

Sputum

The patient was asked to rinse out the mouth with water, then to cough deeply to expectorate a sample of lower respiratory secretions. Early morning sputum sample was collected[63].

Bronchoalveolar lavage (BAL)

A flexible bronchoscope is inserted and a segment of lung was washed with 30 to 50 ml of sterile physiologic saline. The saline was then aspirated, allowing recovery of cellular and non-cellular components of the epithelial surface of the lower respiratory tract[64].

Pleural fluid

The pleural fluid was aspirated by thoracocentesis and collected in a sterile tube.

Ascitic fluid

Under strict aseptic precautions, ascitic fluid was collected and received in a sterile test tube.

Cerebrospinal fluid (CSF)

Under sterile precautions lumbar puncture was done by the physician and CSF collected in a sterile test tube.

Oral scrapings

The oral lesions were scraped with a sterile cotton swab.

Nail

Friable material was removed from under the nail or clippings were taken from the distal border using nail clippers[49].

Collection of swabs

The wound surface was cleaned well with normal saline prior to the collection of swab.Two swabs were taken from the depth of the wound, one for Gram stain and another for culture.

Blood

Under strict aseptic precautions 5-10 ml blood was collected by venipuncture and inoculated into 50ml Brain heart infusion (BHI) broth immediately.

Faeces

Specimens were collected in clean wide mouthed containers.

PROCESSING OF SAMPLES

MACROSCOPIC EXAMINATION

The samples were initially examined macroscopically as follows.

TABLE II: MACROSCOPIC EXAMINATION

Urine	Observed for turbidity and color
Sputum	Examined whether the specimen is purulent, mucopurulent, mucoid, mucosalivary or blood stained
Body fluids (ascitic fluid, CSF)	Examined for turbidity, color, presence of blood or pus
Faeces	Looked for consistency, presence of mucus flakes, pus and blood

MICROSCOPIC EXAMINATION

Direct microscopy

The collected samples were subjected to the following direct microscopic examinations:

a) Wet mount

b) Gram staining

c) Ziehl-Neelsen technique – for Acid Fast Bacilli (AFB)

d) 10% potassium hydroxide mount (KOH)

   A drop of 10% KOH was added to a small amount of sample on the slide and mixed gently and covered with a coverslip. The preparation was examined after half an hour.

e) India Ink preparation for CSF samples

   One drop of CSF was placed on a clean glass slide and mixed with a drop of India Ink and covered with a cover slip. The preparation was examined under low power objective for the presence of capsulated budding yeast cells.

CULTURE

Culture media

Bacterial Culture: The samples were inoculated on MacConkey agar, Blood agar, and Chocolate agar and incubated at 37^0C for 24 hours. Urine samples were inoculated on CLED and BAP. For faeces samples inoculation was also done in XLD. For suspected cases of Tuberculosis samples were also inoculated in LJ media. BHI broth and Selenite F broths were used as enrichment broths for blood and faeces samples respectively.

[BAP – Blood Agar Plate, CAP – Chocolate Agar Plate, LJ – Lowenstein Jensen media, MAC – MacConkey Agar, XLD – Xylose Lysine Deoxycholate media, CLED- Cysteine Lactose Electrolyte Deficient media.]

Fungal Culture: Fungal culture was done in Sabouraud's dextrose agar (SDA).

- For isolating Mycobacterium tuberculosis, the samples were inoculated into two LJ slopes and incubated at 35^0C - 37^0C for 8 weeks. The slopes were examined daily for 1 week for any rapid growers and thereafter at weekly intervals for evidence of growth.
- For fungal culture two SDA slopes were inoculated with the sample and one tube each were incubated at 25^0C and 37^0C.

Processing of Urine samples

A semi quantitative calibrated loop technique was used for the primary isolation of the organism from urine. A loopful of well mixed uncentrifuged urine was streaked onto the surface of blood agar and CLED (Cysteine Lactose Electrolyte Deficient) agar media and incubated at 37^0C for 24 to 48 hrs. Significant bacteriuria was defined as the presence of 10^5Colony Forming Units/ml (CFU/ml). The isolates were identified based on colony morphology on the culture plates, Gram staining, motility and by standard biochemical tests.

INTERPRETATION

INTERPRETATION OF BACTERIAL CULTURES

Bacterial isolates were identified by means of colony morphology, Gram staining, motility and biochemical reactions by standard microbiological techniques as recommended by Clinical and Laboratory Standards Institute (CLSI).

INTERPRETATION OF FUNGAL CULTURE

Inoculated SDA slants were incubated at 25^0C and 35^0C for a minimum of 4 weeks before discarding as culture negative. These slants were inspected daily during the first week and twice weekly during the next three weeks for growth.

Speciation of *Candida*

The *Candida* isolates were speciated by Gram staining, Germ tube test, color of colonies on CHROMagar**,** and sugar fermentation reactions[37].

a) Germ Tube Test

A single colony of *Candida* touched lightly with a loop and emulsified in 0.5 ml of serum in a test tube. Incubated at 37^0C for 2- 4 hours. The germ tubes were seen as long tube like projections extending from the yeast cells[49].

b) Carbohydrate fermentation test

1% yeast extract was added to the carbohydrate fermentation medium with 2% test sugars. The tubes were incubated at 30^0 C for 24-48 hours. The results were usually obtained within 7 days[41].

c) CHROMagar Candida medium

The *Candida* isolates were streaked in CHROMagar and incubated at 30^0Cfor 48-72 hours. Then colony morphology and color produced were observed.

Candida albicans- light green

Candida dubliniensis- dark purple

Candida glabrata- pink to purple

Candida krusei- pink

Candida parapsilosis- cream to pale pink

Candida tropicalis- blue with pink halo

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antibiotic susceptibility testing was performed by the Kirby Bauer method on Mueller Hinton agar (Himedia) according to CLSI protocols. The diameters of zones of inhibition were interpreted according to CLSI standards for each organism. Using standard strains culture media and antibiotic discs were tested for quality control.

The following standard strains were used.

a) *Staphylococcus aureus* – ATCC 25923

b) *Escherichia coli* – ATCC 25922

c) *Pseudomonas aeruginosa* – ATCC 27853

The panel of antibiotics included in the antimicrobial sensitivity testing for Gram negative bacilli were as follows.

TABLE III: ANTIMICROBIAL DISCS FOR GRAM NEGATIVE BACILLI

Antimicrobial	Disc content	Inhibition Zone		
		Resistant	Intermediate	Sensitive
Amikacin	30 µg	14	15 – 16	17
Gentamicin	10 µg	12	13 – 14	15
Cotrimoxazole	1.25 / 23.75 µg	10	11 – 15	16
Ofloxacin	5 µg	12	13 – 15	16
Ciprofloxacin	5 µg	15	16 – 20	21
Cefotaxime	30 µg	14	15 – 22	23
Ceftazidime	30 µg	14	15 – 17	18
Nitrofurantoin	300 µg	14	15 – 16	17
Norfloxacin	10 µg	12	13 – 16	17
Piperacellin / Tazobactum	100 / 10 µg	17	18 – 20	21
Imipenem	10 µg	13	14 – 15	16
Ampicillin	10 µg	13	14 – 16	17
Chloramphenicol	30 µg	12	13-17	18

The panel of antibiotics included in the antimicrobial sensitivity testing for Gram positive cocci were as follows.

TABLE IV: ANTIMICROBIAL DISCS FOR GRAM POSITIVE COCCI

Antimicrobial	Disc content	Inhibition Zone		
		Resistant	Intermediate	Sensitive
Amikacin	30 µg	14	15 – 16	17
Erythromycin	15 µg	13	14 – 22	23
Cotrimoxazole	1.25 / 23.75 µg	10	11 – 15	16
Cephalexin	30 µg	14	15 - 17	18
Ciprofloxacin	5 µg	15	16 – 20	21
Amoxycillin / Clavulanic acid	20 / 10 µg	19	-	20
Penicillin	10 units	28	-	29

Oxacillin (1 µg) disc was used to detect MRSA. Broth macrodilution method was done to obtain MIC for Vancomycin. MIC ≤ 2 µg / ml for vancomycin was reported as sensitive[12].

A) DETECTION OF β LACTAMASE ENZYME PRODUCTION IN GRAM NEGATIVE BACILLI[12]

1) SCREENING METHOD

Isolate of gram negative bacilli with the following resistance pattern by disc diffusion method was considered to be possible ESBL producing strain.

TABLE V: ZONE DIAMETER FOR ESBL PRODUCING STRAIN

Antibiotic	Zone diameter for possible ESBL producing strain
Ceftazidime (30μg)	≤ 22mm
Cefotaxime(30 μg)	≤ 27mm
Ceftriaxone(30 μg)	≤ 25mm
Aztreonam (30 μg)	≤ 27 mm

2) CLSI PHENOTYPIC CONFIRMATION METHOD

With a sterile bacteriological loop 3-5 identical colonies were picked from a fresh culture and inoculated into 5 ml of nutrient broth. The broth was incubated at 35°C for 2-4 hrs. The turbidity was matched with 0.5 McFarlands standard. Lawn culture of the organism to be tested was made on MHA plate (Himedia, Mumbai). Antibiotic discs Ceftazidime (CAZ 30μg) and Ceftazidime/ Clavulanic acid (CAZ/ CA 30μg/ 10μg) (Himedia, Mumbai) were placed on the plate and incubated at 35°C overnight. Organism with ≥5mm increase in zone diameter for Ceftazidime tested in combination with Clavulanic acid versus its zone when tested alone confirms an ESBL producing organism.

3) DOUBLE DISK DIFFUSION SYNERGY TEST

In this test discs of third generation cephalosporins (ceftazidime and cefotaxime) and Augmentin (20μg/10μg) (Himedia, Mumbai) were kept 30 mm apart from center to center on inoculated Muller-Hinton Agar (MHA). The test organism was considered to be an ESBL producer, if the zone size around the cephalosporin disc increases towards the augmentin disc.

4) TRIPLE ESBL DETECTION USING EZY MIC^{TM} STRIP (HIMEDIA)

The organism to be tested was swabbed onto a Muller-Hinton Agar (MHA) plate. Ezy MIC^{TM} strip was placed on the inoculated agar plate and incubated at 37^0C overnight. MIC was interpreted as the point of intersection of the inhibition ellipse with E Test strip edge. Ratio of Cephalosporin MIC and Cephalosporin Clavulanic acid MIC $\geq$ 8 indicates positive result.

B) DETECTION OF METHICILLIN RESISTANCE IN *Staphyloccus aures*

1) DISC DIFFUSION METHOD

A lawn culture of the Staphylococcal culture was made on the MHA plate and Oxacillin disc was applied and incubatedat 35° C for 24 hours in ambient air. According to CLSI criteria with 1μg Oxacillin disc, diameters of $\leq$ 10, 11-12, $\geq$ 13 mm corresponded to categorization as resistant, intermediate or susceptible[12].

2) OXACILLIN SCREEN AGAR

Oxacillin screen agar was performed by direct colony suspension method. The inoculum was adjusted to match a turbidity of 0.5 McFarland standard. Then the

suspension was inoculated on Mueller-Hinton agar plate containing 4% NaCl and with 6µg/ml of Oxacillin and incubated at 35°C for 24 hours. If growth was seen on the plates, the organism was considered as methicillin resistant.

C) MINIMUM INHIBITORY CONCENTRATION (MIC) FOR DETECTING VANCOMYCIN RESISTANCE

- **Culture media used**

Mueller Hinton broth. (pH 7.2-7.4)

- **Preparation of stock antibiotic solution**

Antibiotic stock solution was prepared using the formula

$$W = \frac{1000}{p} \times V \times C$$

where

p=potency of the antibiotic in relation to the base. (For vancomycin, p= 950/1000 mg; Himedia)

V = volume of the stock solution to be prepared (10ml)

C =final concentration of the antibiotic solution (1024µg/ml)

W = weight of the antibiotic to be dissolved in the volume V

- Antibiotic dilutions were prepared from concentration of 32µg/ml to 0.125µg/ml.

- **Inoculum preparation for the test organism and ATCC control and incubation**
    - ✓ To 9.9 ml of MH broth 0.1 ml of 0.5 McFarland turbidity matched test organism broth was added and mixed well.
    - ✓ 1 ml of this inoculum was transferred to each tube containing antibiotic dilutions and also to the control tube.
    - ✓ The procedure was repeated for ATCC control strains
    - ✓ Incubated at 37^0C overnight and then reading was taken.
    - ✓ The lowest concentration of the antibiotic at which there is no visible growth will be the MIC for the test organism.

ANTIFUNGAL SUSCEPTIBILITY TESTING BY MICRODILUTION METHOD

Antifungal susceptibility testing was done according to the CLSI M-38A document for filamentous fungi and CLSI M-27A2 document for yeast.

PROCEDURE

GROWTH MEDIUM

The growth medium used was RPMI-1640 (Rosewell Park Memorial Institute-1640) which is a synthetic medium and is supplemented with 0.3 l-glutamate/L without sodium bicarbonate. It is buffered at the pH of 7.0- 7.2 using MOPS(3-N-morpholinopropane sulfonic acid).

DRUG DILUTION PREPARATION

The drug dilutions were prepared as described in the CLSI M38-A document.

INOCULATION IN RPMI-1640 MEDIUM AND INTERPRETATION

The inoculation was done in sterile 96-well microtitre plate. 100µl of the conidial suspension/ yeast suspension was inoculated into each well. Then 100µl of diluted drug was added correspondingly to each well. Controls were also inoculated and microtitre plates were incubated at 35^0C for 48 hours and then reading was taken. The growth in each well was compared with that of the growth control with a reading mirror. A numerical score is given to each well as follows:

4 – no reduction in growth

3 – slight reduction in growth or 75% of growth control

2 – prominent reduction in growth or 50% of growth control

1 – slight growth or 25% of growth control

0 – optically clear or absence of growth

ANTINUCLEAR ANTIBODY (ANA) DETECTION BY INDIRECT IMMUNOFLOURESCENCE METHOD

The Kit usedis: HEp-2 slides (Biocientifica S.A)

PROCEDURE

a) The serum samples were diluted as 1:10 and 1:40
b) The diluted samples and controls were added to the slides to cover the reactive areas.
c) Incubated in a moist chamber for 30 minutes at room temperature.
d) The slides were washed three times in a trough containing Phosphate Buffered Saline (PBS) and wiped with filter paper keeping the reactive areas wet.

e) Each reactive area covered immediately with diluted human Anti-IgG FITC and incubated in a moist chamber for 30 min at room temperature.

f) Step 4 was repeated.

g) The reactive areas were covered with Evan's blue and the excess stain washed with PBS, wiped with filter paper.

h) The mounting fluid added immediately and examined under immunoflourescence microscope.

DETECTION OF ANTIBODIES TO ds DNA BY IgG ELISA

(The Calbiotech, Inc. (CBI) ds DNA IgG ELISA Kit)

a) 10 µl of the sample added to 200 µl of sample diluent to prepare 1:21 dilution.

b) 100 µl of diluted sera, calibrator and controls were dispensed into the appropriate wells. Incubated for 20 minutes at room temperature.

c) The liquids were removed from all wells and washed three times with 300 µl of 1X wash buffer and blotted on absorbance paper.

d) 100 µl of enzyme conjugate dispensed to each well and incubation done for 20 minutes at room temperature.

e) Enzyme conjugate was removed from all wells. Washed the wells three times with 300 µl of 1X wash buffer and blotted on absorbance paper.

f) Dispensed 100 µl of TMB substrate and incubated for 10 minutes at room temperature.

g) Added 100 µl of stop solution.

h) O.D. read at 450 nm using ELISA reader within 15 min.

STUDY OF COMPLEMENT LEVELS (C3 and C4)

The kit used is Diffu-Plate (Biocientifica S.A)

This kit is used for measurement of human C3 and C4 levels in serum and other biological fluids. It uses the principle of radial immunodiffusion (RID) and involves diffusion of antigen radially from a cylindrical well through an agarose gel which contains the appropriate monospecific antibody. Antigen antibody complexes are formed which will form a precipitin ring. Serum samples from all SLE patients were subjected to quantitative analysis of Complement levels by the following procedure.

EQUIPMENTS REQUIRED

1) RID plate for 12 tests packed in foil pouch
2) Micropipettes
3) Control sera
4) Ruler capable of measuring with 0.1 mm precision
5) Reference table showing the correspondence between diameters and concentrations

PROCEDURE

1) The RID plates were opened and kept at room temperature for 5 minutes.
2) Wells were filled with 5 µl of test serum or control serum. A wet gauze was placed in the center of the plate to avoid agarose dehydration and plates were closed tightly.
3) The plates were allowed to stay flat at room temperature for 48 hours.
4) The ring diameters were measured at the end of incubation.
5) The results were read from the RID reference table.

RESULTS

A total of 110 SLE patients with clinically suspected infections were studied.

TABLE 1: AGE-SEX DISTRIBUTION OF SLE PATIENTS

n=110

Gender →	Male		Female		Total	
Age Range ↓	**No.**	**Percentage**	**No.**	**Percentage**	**No.**	**Percentage**
0 – 10(n=1)	1	100.00%	0	0.00%	1	0.91%
11 – 20(n=28)	3	10.71%	25	89.29%	28	25.45%
21 – 30(n=58)	4	6.90%	54	93.10%	58	52.73%
31 – 40(n=17)	3	17.65%	14	82.35%	17	15.45%
41 – 50(n=5)	1	20.00%	4	80.00%	5	4.55%
51 – 60(n=1)	0	0.00%	1	100.00%	1	0.91%
Total	**12**	10.91%	**98**	89.09%	**110**	100.00%

- Out of 110 SLE patients included in the present study 98 were female and 12 were male. There was a female preponderance with a female:male ratio of 8:1.
- 52.73 % of the patients were in the age group of 21 to 30 years.
- The mean age of male and female SLE patients were 24.83 and 25.68 respectively.
- The mean age at diagnosis of male and female SLE patients were 24.83 and 25.68 respectively.
- The average duration of SLE was 37 months (3.08 years).

TABLE 2: CHILDHOOD SLE

n = 15

Gender	No.	Percentage
Male	2	13.33%
Female	13	86.67%
Total	**15**	

Among the 110 SLE patients, 15 patients (13.6%) were children. Mean age of onset of disease in childhood lupus was 13 years and female:male ratio was 6.5:1

TABLE 3: FREQUENCY OF VARIOUS CLINICAL MANIFESTATIONS IN SLE PATIENTS

n=110

Clinical Manifestations	Number	Percentage
Arthritis	73	66.36%
Fever	54	49.09%
Skin rash	49	44.55%
Anaemia	48	43.64%
Oral ulcers	46	41.82%
Renal involvement	45	40.91%
Alopecia	31	28.18%
Malar rash	30	27.27%
Neuropsychiatric Manifestation	28	25.45%
Photosensitivity	27	24.55%
Cardiovascular Involvement	9	8.18%
Past History of Tuberculosis	8	7.27%
Antiphospholipid antibody syndrome	7	6.36%
Hypothyroidism	5	4.55%
Pleural effusion	5	4.55%

Arthritis was the common clinical manifestation seen in 73 (66.36%) patients followed by fever 54 (49.09%), skin rash 49 (44.55%) and anaemia 48 (43.64%).

TABLE 4: DISTRIBUTION OF PATIENTS WITH LUPUS NEPHRITIS (LN)

n = 45

Patients →→→ **Lupus Nephritis Class ↓**	**No.**	**Percentage**
Lupus Nephritis - Class I (n=6)	6	13.33%
Lupus Nephritis - Class II (n=10)	10	22.22%
Lupus Nephritis - Class III (n=7)	7	15.56%
Lupus Nephritis - Class IV (n=19)	19	42.22%
Lupus Nephritis - Class V (n=3)	3	6.67%
Total	**45**	

- Among the 110 SLE patients, 45 patients had renal involvement in the form of Lupus Nephritis.
- The most common class of Lupus Nephritis (LN) was Class 4 (42.22%) followed by Class 2 LN (22.22%).
- The frequencies of other LN classes were LN Class 3 (15.56%), LN Class 1 (13.33 %) and LN Class 5 (6.67%).

TABLE 5: ANALYSIS OF DISTRIBUTION OF COLLECTED SAMPLES

n=150

	Samples Collected	
Type of sample ↓	**No.**	**Percentage**
Urine	84	56.00%
Sputum	22	14.67%
Blood	21	14.00%
Pus	8	5.33%
Ear Swab	2	1.33%
Oral scrapings	2	1.33%
Ascitic fluid	1	0.67%
BAL	1	0.67%
Nail scraping	1	0.67%
CSF	3	2.00%
Faeces	5	3.33%
Total	**150**	**100.00%**

From the 110 SLE patients the main samples collected were urine 84 (56%), sputum 22 (14.67%) and blood 21 (14%). The rest of the samples are mentioned in the above tabular column.

TABLE 6: FREQUENCY OF PATIENTS WITH AND WITHOUT INFECTION

n=110

Patients distribution ↓↓↓	**No.**	**Percentage**
No. of Patients with Infection	60	54.55%
No. of Patients without Infection	50	45.45%
Total	**110**	**100.00 %**

Among the 110 SLE patients, 60 (54.55%) had infectious episodes.

TABLE 7: GENDER DISTRIBUTION OF PATIENTS WITH AND WITHOUT INFECTION

n=110

Gender →→→	**Male**		**Female**	
Patients ↓↓↓	**No.**	**Percentage**	**No.**	**Percentage**
Patients with infection (n=60)	7	11.67%	53	88.33%
Patients without infection (n=50)	5	10.00%	45	90.00%

Out of 110 SLE patients, 60 patients had infection. Among them 53 were female and 7 were male. Out of the 60 patients with infection, 3 died due to infections like pneumonia and sepsis.

TABLE 8: EPISODES OF INFECTION IN SLE PATIENTS

n=60

Number of episodes	**One**	**Two**	**Three**
No. of Patients	51	8	1

Among the 60 patients with infection, 51 patients had one episode of infection whereas 8 patients had 2 episodes and 1 patient had 3 episodes of infection.

TABLE 9: DISTRIBUTION OF SAMPLES WITH SIGNIFICANT GROWTH

n=74

Sites Of Infection	Significant growth	
	No.	**Percentage**
Urinary Tract	40	54.05%
Respiratory Tract (Sputum/BAL)	12	16.22%
Blood	9	12.16%
Skin and Soft Tissue(Pus, nail scraping)	8	10.81%
Ear	2	2.70%
Oral Cavity	2	2.70%
Peritoneum	1	1.35%
Total	**74**	

Out of 150 samples collected, significant growth was found in 74 samples. Infections were common in urinary tract (54.05%). Next significant growth was found in respiratory tract 12 (16.22%). Other sites of infection were mentioned in the above table in the decreasing order of frequency. No significant growth was obtained from samples of CSF and faeces.

TABLE 10: DISTRIBUTION OF ISOLATED ORGANISMS

Bacterial Isolates

Bacteria Isolated	Number
Escherichia coli	20
Pseudomonas aeruginosa	14
Klebsiella pneumoniae	12
Staphylococcus aureus	10
Staphylococcus epidermidis	3
Acinetobacter baumannii	2
Enterococcus faecalis	2
Proteus mirabilis	2
Citrobacter freundii	1
Klebsiella oxytoca	1
Salmonella typhi	1
Total	**68**

Fungal Isolates

Fungi Isolated	Number
Candida albicans	4
Candida tropicalis	1
Aspergillus flavus	1
Total	**6**

Bacterial infections were predominant than fungal infections in patients with SLE.

TABLE 11: DISTRIBUTION OF ORGANISMS ISOLATED FROM URINARY TRACT

n=40

Organism Isolated	Number	Percentage
Gram Negative Bacteria		
Escherichia coli	20	50.00
Pseudomonas aeruginosa	4	10.00
Klebsiella pneumoniae	2	5.00
Proteus mirabilis	2	5.00
Citrobacter freundii	1	2.50
Klebsiellaoxytoca	1	2.50
Acinetobacter baumannii	1	2.50
Gram Positive Bacteria		
Staphylococcus aureus	4	10.00
Enterococcus faecalis	2	5.00
Staphylococcus epidermidis	1	2.50
Yeast		
Candida albicans	1	2.50
Candida tropicalis	1	2.50
Total	**40**	

Out of 84 urine samples processed significant growth was present in 40 samples. *Escherichia coli* was the predominant Gram negative bacteria isolated from 20 (50%) samples. Among Gram positive bacteria, *Staphylococcus aureus* was the common isolate from 4 (10%) patients.

TABLE 12: DISTRIBUTION OF ORGANSISMS ISOLATED FROM RESPIRATORY TRACT

n=12

Organism Isolated	Number	Percentage
Gram Negative Bacteria		
Klebsiella pneumoniae	8	66.67%
Pseudomonas aeruginosa	2	16.67%
Gram Positive Bacteria		
Staphylococcus aureus	1	8.33%
Staphylococcus epidermidis	1	8.33%
Total	**12**	

A total of 23 samples were processed from respiratory tract, out of which 12 samples showed significant growth.

Klebsiella pneumoniae was the predominant organism isolated from 8 (66.67%) patients.

TABLE 13: DISTRIBUTION OF ORGANISMS ISOLATED FROM BLOOD

n=9

Organism Isolated	Number	Percentage
Gram Negative Bacteria		
Klebsiella pneumoniae	2	22.22%
Pseudomonas aeruginosa	2	22.22%
Salmonella typhi	1	11.11%
Acinetobacter baumannii	1	11.11%
Gram Positive Bacteria		
Staphylococcus aureus	2	22.22%
Staphylococcus epidermidis	1	11.11%
Total	**9**	

A total of 9 organisms were isolated from 21 samples of blood processed. Out of the 6 Gram negative bacteria isolated, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* constituted (2 patients) 22.22% each. Among Gram positive bacteria, *Staphylococcus aureus* was the predominant isolate.

TABLE 14: DISTRIBUTION OF ORGANISMS ISOLATED FROM SKIN AND SOFT TISSUE

n = 8

	No. of Organisms	**Percentage**
Pseudomonas aeruginosa	4	50.00%
Staphylococcus aureus	3	37.50%
Candida albicans	1	12.50%
Total	8	

Among the 9 samples processed (pus-8, oral scraping-1) 8 organisms were isolated from infections of skin and soft tissue. Out of which *Pseudomonas aeruginosa* constituted 50%, followed by *Staphylococcus aureus* 37.5% and *Candida albicans* 12.5%.

TABLE 15: DISTRIBUTION OF ORGANISMS ISOLATED FROM OTHER SITES

Organism / System	***Pseudomonas aeruginosa***	***Candida albicans***	***Aspergillusflavus***
Ear	1	-	1
Oral Cavity	-	2	-
Peritoneum	1	-	-

a) *Aspergillus flavus* and *Pseudomonas aeruginosa* one each were isolated from ear infection.
b) *Candida albicans* was isolated from oral cavity of 2 patients with oral thrush.
c) *Pseudomonas aeruginosa* was the causative agent of peritonitis in one patient with SLE.

TABLE 16: ANALYSIS OF VARIOUS SITES OF INFECTIONS IN PATIENTS WITH LUPUS NEPHRITIS

n=45

Patients →→→	Infected		Not Infected	
Lupus Nephritis Class ↓	**No.**	**%**	**No.**	**%**
Lupus Nephritis - Class I (n=6)	0	0.00%	6	100.00%
Lupus Nephritis - Class II (n=10)	8	80.00%	2	20.00%
Lupus Nephritis - Class III (n=7)	2	28.57%	5	71.43%
Lupus Nephritis - Class IV (n=19)	15	78.95%	4	21.05%
Lupus Nephritis - Class V (n=3)	2	66.67%	1	33.33%

- Among the 45 patients with LN, infections were present in 27 (60%) patients and Urinary tract was the common site of infection involved in 17 (62.96%) patients.
- Out of the 45 SLE patients with LN, 6 patients were on haemodialysis.

TABLE 17: FREQUENCY OF SITES INVOLVED IN VARIOUS CLASSES OF LUPUS NEPHRITIS

n=27

System →→→	Urinary Tract		Blood		Respiratory Tract		Skin and Soft Tissue		Peritoneum	
Lupus Nephritis patients with infection ↓↓↓	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Lupus Nephritis - Class 1 (n=0)	0	-	0	-	0	-	0	-	0	-
Lupus Nephritis - Class 2 (n=8)	5	62.50	1	12.50	1	12.50	1	12.50	0	0.00
Lupus Nephritis - Class 3 (n=2)	2	100.00	0	0.00	0	0.00	0	0.00	0	0.00
Lupus Nephritis - Class 4 (n=15)	9	60.00	2	13.33	2	13.33	1	6.67	1	6.67
Lupus Nephritis - Class 5 (n=2)	1	50.00	1	50.00	0	0.00	0	0.00	0	0.00
Total	**17**	**62.96**	**4**	**14.81**	**3**	**11.11**	**2**	**7.41**	**1**	**3.70**

- Out of 27 Lupus Nephritis patients with infection, 17 (62.96%) patients had urinary tract infection.
- The other less frequently involved sites in Lupus Nephritis patients were blood, respiratory tract, skin and soft tissue, and peritoneum.
- The sites of infection are similar in patients with and without lupus nephritis.

TABLE 18: ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACTERIA FROM URINARY TRACT

n=31

Organism	AK		GENTA		COTRI		CEF		CZ		PT		FU		NF		IMI	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Escherichia coli **(n=20)**	18	90	10	50	6	30	5	25	4	20	19	95	14	70	6	30	20	100
Pseudomonas aeruginosa **(n=4)**	3	75	2	50	2	50	2	50	2	50	3	75	1	25	2	50	4	100
Proteus mirabilis **(n=2)**	2	100	2	100	2	100	2	100	2	100	2	100	1	50	2	100	2	100
Klebsiella pneumoniae **(n=2)**	1	50	1	50	2	100	2	100	2	100	2	100	1	50	1	50	2	100
Klebsiella oxytoca **(n=1)**	1	100	0	0	0	0	0	0	0	0	1	100	1	100	0	0	1	100
Citrobacter freundii **(n=1)**	1	100	1	100	0	0	0	0	0	0	1	100	0	0	0	0	1	100
Acinetobacter baumannii **(n=1)**	1	100	1	100	1	100	1	100	1	100	1	100	1	100	0	0	1	100

[AK – Amikacin; GENTA – Gentamicin; COTRI – Cotrimoxazole; CEF – Cefotaxime;

CZ – Ceftazidime; PT – Piperacillin / Tazobactum; FU – Nitrofurantoin; NF – Norfloxacin; IMI – Imipenem]

- The organisms showed resistance pattern to wide range of antibiotics like Cotrimoxazole, Nitrofurantoin, Norfloxacin and third generation Cephalosporins.
- All the gram negative bacteria isolated from urinary tract were 100% sensitive to Imipenem.

TABLE 19: ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACTERIA FROM SITES OTHER THAN URINARY TRACT

n=21

Drug →→→	AK		GENTA		CIP		OF		CEF		CZ		PT		IMI	
Organism ↓↓↓	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
***Klebsiella pneumoniae* (n=10)**	9	90	8	80	7	70	2	20	6	60	6	60	10	100	10	100
***Pseudomonas aeruginosa* (n=10)**	6	60	3	30	7	70	5	50	2	20	3	30	9	90	10	100
***Acinetobacter baumannii* (n=1)**	1	100	0	0	1	100	1	100	1	100	1	100	1	100	1	100

[AK – Amikacin; GENTA – Gentamicin; CIP - Ciprofloxacin; OF – Ofloxacin;

CEF – Cefotaxime; CZ – Ceftazidime; PT – Piperacillin / Tazbactum; IMI –Imipenem]

- A total of 21 Gram Negative Bacteria were isolated from various sites like respiratory tract, blood, skin and soft tissue, ear and peritoneum.

- The organisms showed significant resistance to Cephalosporins and Fluoroquinolones.

- One isolate of *Salmonella typhi* was found sensitive to all the antibiotics tested like Ampicillin, Ciprofloxacin, Gentamicin, Cotrimoxazole, Chloramphenicol and Ceftazidime.

TABLE 20: ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF GRAM POSITIVE COCCI FROM URINARY TRACT

n=6

Drug →→→	AK		COTRI		ERY		CIP		CEP		AC		VAN (MIC)	
Organism ↓↓↓	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Staphylococcus aureus	4	100	3	75	0	0	0	0	0	0	4	100	4	100
Enterococcus faecalis	1	50	0	0	0	0	1	50	0	0	0	0	2	100
Staphylococcus epidermidis	1	100	1	100	0	0	1	100	1	100	1	100	1	100

[AK – Amikacin; COTRI – Cotrimoxazole; ERY – Erythromycin, CIP – Ciprofloxacin;

CEP – Cephalexin; AC – AmoxycillinClavulanate; VAN – Vancomycin]

- All the six gram positive cocci from urinary tract were sensitive to Vancomycin by MIC method. (MIC < 2 µg/ml)

TABLE 21: ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF GRAM POSITIVE COCCIFROM SITES OTHER THAN URINARY TRACT

n=8

Drug →→→	AK		COTRI		ERY		CIP		CEP		PEN		AC		VAN	
Organism ↓↓↓	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Staphylococcus aureus (n=6)	6	100	5	83.3	2	33.3	4	66.7	2	33.3	1	16.7	4	66.7	6	100
Staphylococcus epidermidis (n=2)	2	100	1	50	1	50	2	100	1	50	2	100	2	100	2	100

[AK – Amikacin; COTRI – Cotrimoxazole; ERY – Erythromycin, CIP – Ciprofloxacin;

CEP – Cephalexin; PEN – Penicillin; AC – AmoxycillinClavulanate; VAN – Vancomycin]

- A total of eight gram positive cocci were isolated from various sites like blood, respiratory tract, and skin and soft tissue.
- All of them were found sensitive to Vancomycin by MIC. (MIC < 2µg/ml)

TABLE 22: DISTRIBUTION OF ESBL PRODUCING ORGANISMS

Organism	No.	Percentage
Escherichia coli (n=20)	15	75.00%
Pseudomonas aeruginosa (n=14)	9	64.29%
Klebsiella pneumoniae (n=12)	4	33.33%
Citrobacter freundii (n=1)	1	100.00%
Klebsiella oxytoca (n=1)	1	100.00%

- Out of the 53 gram negative bacteria isolated, 30 were ESBL producers.
- *Escherichia coli* was the predominant ESBL producer. (15 out of 20)

TABLE 23: DETECTION OF MRSA AMONG THE ISOLATES OF *Staphylococcus aureus*

n=10

Organism	Disc diffusion method	Oxacillin salt agar
Staphylococcus aureus	8 (100 %)	8 (100 %)

- Among the ten isolates of *Staphylococcus aureus*,8 were found to be MRSA by both disc diffusion method and Oxacillin salt agar method.

TABLE 24: ANTIFUNGAL SUSCEPTIBILITY OF FUNGI BY BROTH MICRODILUTION METHOD

Drugs →→→	**AMB**	**ITRA**	**FLU**	**VORI**
Organisms ↓↓↓				
Candida albicans (n=4)	S	S	S	S
Candida tropicalis (n=1)	S	S	S	S
Aspergillus flavus (n=1)	S	S	-	S

AMB – Amphotericin B; ITRA – Itraconazole; FLU – Fluconazole; VORI - Voriconazole

All the fungi isolated were sensitive to the antifungals tested by broth microdilution method.

The MIC of AMB, ITRA, VORI for *Candida spp.* was ≤ 1 µg/ml and ≤ 8 µg/ml for FLU.

The MIC of AMB was ≤ 2 µg/ml and ≤ 8 µg/ml for ITRA and VORI when testing for *Aspergillus flavus.*

TABLE 25: ANALYSIS OF IMMUNOLOGICAL PARAMETERS IN SLE PATIENTS

n= 110

Immunological Parameters	No. of patients	Percentage
Positive ANA	108	98.18%
Positive Anti-ds DNA	77	70.00%
Both ANA and Anti-ds DNA Positive	77	70.00%

Among 110 SLE patients, ANA was positive in 108 (98.18 %) patients and Anti-ds DNA was positive in 77 (70%) patients. 77 patients with positive Anti-ds DNA were also positive for ANA.

TABLE 26: ANALYSIS OF ANA PATTERNS IN SLE PATIENTS BY INDIRECT IMMUNOFLOURESCENCE

n= 108

ANA Patterns	No.	Percentage
Homogenous	56	51.85%
Speckled	28	25.93%
Rim	24	22.22%
Total	**108**	

The ANA patterns were studied using Hep-2 slides by Indirect Immunoflourescence method. Homogenous pattern was the common pattern present in 56 (51.85%) patients followed by Speckled pattern and Rim pattern.

TABLE 27: ANALYSIS OF C3 / C4 LEVELS IN SLE PATIENTS

n= 110

C3 / C4	No.	Percentage
Low C3	70	63.64%
Low C4	61	55.45%
Low C3 and C4	60	54.55%

Normal values of C3 and C4

C3: 80- 160 mg/dl

C4: 20- 40 mg/ dl

- By RID method C3 and C4 levels were analyzed in 110 SLE patients and it was found that C3 Levels were decreased in 70 (63.64%) patients and C4 levels were decreased in 61 (55.45%) patients.

- Both C3 and C4 levels were decreased in 60 (54.55%) patients.

TABLE 28: STEROID VS. INFECTION

n = 110

	Total no. of patients	Infected		Not Infected	
Daily Prednisolone dosage ↓		**No.**	**%**	**No.**	**%**
< 20 mg	74	31	41.89%	43	58.11%
≥ 20 mg	36	29	80.56%	7	19.44%
Total	**110**	**60**		**50**	

Daily Prednisolone dosage ≥ 20 mg was associated with an increased risk of infection with a p value 0.0003. (<0.05 which is statistically significant)

TABLE 29: CYCLOPHOSPHAMIDE + STEROID VS. INFECTION

n = 110

		Infected		Not Infected	
	Total no. of patients	**No.**	**%**	**No.**	**%**
No. of patients on Cyclophosphamide + Prednisolone	29	22	75.86%	7	24.14%
No. of patients not on Cyclophosphamide	81	39	48.15%	42	51.85%
Total	**110**	**61**		**49**	

The risk of infection was higher in patients receiving Cyclophosphamide + Prednisolone with a p value of 0.0183 (p value <0.05 which is statistically significant)

TABLE 30: MMF VS. INFECTION

n=110

		Infected		Not Infected	
	Total no. of patients	**No.**	**%**	**No.**	**%**
No. of patients on Mycophenolatemofetil	8	4	50.00%	4	50.00%
No. of patients not on Mycophenolatemofetil	102	57	55.88%	45	44.12%
Total	**110**	**61**		**49**	

- Out of the 110 SLE patients included in this study, only 8 were on treatment with MMF.
- Among the 8 patients on MMF, only 4 developed infectious episodes.
- No association was found between MMF intake and infection with a p value of 0.7472 (p value > 0.05 which is statistically not significant).

DISCUSSION

SLE is a systemic autoimmune disease mainly affecting women of reproductive age group. Due to the various immunological dysfunctions, SLEpatients are inherently at risk for infections. This susceptibility to infections is further increased by the immunosuppressants which are used in the treatment of SLE.

This study was conducted at the Institute of Microbiology, Madras Medical College in association with the Departments of Rheumatology and Nephrology, Rajiv Gandhi Government General Hospital, Chennai, to detect the bacterial and fungal pathogens causing infection in SLE patients. This study also focused on the various clinical manifestations, immunological parameters and association of infection with steroids and other immunosuppressants.

Among the 110 SLE patients included in the study there were 98 female and 12 male. The female:male ratio of SLE patients in the present study was 8:1 [**TABLE:1**]. This female preponderance highlights the role played by sex hormones on the development of SLE. Malaviya *et al*[54]in their study reported a female to male ratio of 8:1. Jacobsen, Petersen *et al,* in a Danish study conducted at Copenhagen on 513 patients also showed a similar female to male ratio of 8:1[35]. The female to male ratio in a multicenter study conducted by Cervera, Khamashta *et al*.[76] over a 5 year period is 10:1. H-AL-Rayes *et al*.[26]in their study showed a lower female to male ratio of 4.4:1.

In this study the median age of onset of SLE was 22 years. Binoy *et al*[69]observed a median age of disease onset at 21.6 years which is in agreement with the present study.

Malaviya *et al*.[54] and Vaidya *et al*[85]have noted a median age of disease onset of 24 and 26 years respectively. This is higher than the age of disease onset of the present study.

Duration of SLE ranged from one month to 19 years with a mean of 37 months. In a study done in Western India on 60 SLE patients, Renu Saigal *et al*[73]noted a mean duration of SLE as 24 months. Malaviya *et al*[54]reported median duration of SLE as 17 months. Both the studies show a lower age of disease onset compared with the present study.

Most of the patients (52.73%) were in the reproductive age group of 21 to 30 years. This is in agreement with the study by Renu Saigal *et al*[73] in which majority of patients were in the age group of 21 to 30 years.

In this study of 110 SLE patients, 15 (13.6%) patients were children (aged below 16 years) which is lower than the earlier reports indicating 15-17% of all SLE patients are children worldwide[18] [**TABLE:2].** The mean age of onset of disease in childhood lupus in the present study was 13 years. Huang *et al*[40] in their study on Paediatric lupus also observed a mean age at diagnosis of 8.6-13.5 years which is comparable to the current study. The female to male ratio in the childhood lupus group in the current study was 6.5:1. Huang *et al* (D 9) also reported a female to male ratio of 6.2:1 in children with SLE.

The common clinical manifestations observed in the study group were arthritis (66.36%), fever (49.09%) and skin rash (44.55%)**[TABLE:3]** which is in agreement with the study done by Kosaraju *et al.*[44] in South Indian SLE patients. The other clinical manifestations observed in the study were anaemia (43.64%), oral ulcers (41.82%), renal

involvement (40.91%), alopecia (28.18%), malar rash (27.27%), neuropsychiatric manifestations (25.45%) and photosensitivity (24.55%). In reports given by Malaviya *et al*.[54] and Alarcon *et al.* [70]renal involvement, malar rash, oral ulcers, neuropsychiatric manifestations and photosensitivity were the common clinical manifestations.

The other associated illnesses in the present study include cardiovascular involvement (9), hypothyroidism (5), pleural effusion (5), history of tuberculosis (8), APS (7), history of abortion (3), retinal vasculitis (2) and avascular necrosis of bone (2).

Two patients gave family history of SLE, out of them mother of one patient had SLE. Another patient had two sisters with SLE, of which one of them died due to SLE related complications. In a study of clinical profile of SLE patients in Western India by Renu Saigal *et al*.[73] family history of SLE was present in 4 patients.

Among the 110 patients included in the study, 45 patients (40.91%) had lupus nephritis**[TABLE:4]**. This is comparable to the study of Madhavan *et al*. [50]involving 54 SLE patients in which renal involvement has been recorded in 38.8% of patients.

In this study, WHO class IV nephritis was the most commonly seen histological pattern seen in 19 (42.22%) patients and this finding is comparable to the following two studies. In a study conducted by Renu Saigal *et al*. [73] on 60 SLE patients, class IV LN was the common histologic pattern observed. Similarly a study by C.C.Mok *et al*. (D29) also recorded LN class IV in 65 % of patients with LN.

The samples collected from SLE patients include urine, sputum, blood, nail scrapings, ear swab, oral scrapings, ascitic fluid, cerebrospinal fluid and

faeces**[TABLE:5].** In this study of 110 SLE patients, 60 (54.55%) patients suffered from infection. de Luis *et al*.[14]in their study which included 96 cases of SLE reported infection in 55% of patients, H-Al-Rayes *et al*.[26]in their retrospective study of 199 SLE patients in Saudi Arabia recorded infection in 58.79% of patients and the present study is comparable with both the above studies.

A total of 74 infections were observed in 60 patients in this study, **[TABLE:6,7]**out of which 51 patients experienced one infectious episode, 8 had two episodes of infection and 1 patient had 3 episodes of infection.**[TABLE:8]** In 4 patients with single episode of infection, two sites were involved.

The most common sites of infection were the urinary tract and the respiratory tract which contributed to 54.05% and 16.22% of all the infections respectively**[TABLE:9]**. This preponderance of urinary and respiratory tract has also been observed in other studies conducted by Ginzler E *et al*. [21], Staples PJ *et al*. [79]and de Luis *et al*. [14]

Other sites involved in the present study were blood (12.16%), skin and soft tissue (10.81%), ear (2.7%), oral cavity (2.7%) and peritoneum (1.35%). de Luis *et al*. [14] reported skin and soft tissue and blood as the third and fourth common sites of infection.

In this study, bacterial infections 93.24% (69 out of 74) were common than fungal infections**[TABLE:10]**. This is in agreement with earlier reports of upto 80% incidence of bacterial infections in SLE patients from India [78], Malaysia [68], Singapore [66], Spain[14] and United States of America [65].

Most frequently isolated uropathogen was *Escherichia coli*(50%) **[TABLE:11],** a result which is similar to that described by C Hidalgo-Tenorio *et al*. [91]in their study in Spain. The other pathogens responsible for urinary tract infection were *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis, Citrobacter freundii, Klebsiella oxytoca, Staphylococcus epidermidis, Acinetobacter baumannii, Candida albicans* and *Candida tropicalis.*

Klebsiella pneumoniae was the frequently isolated organism from respiratory tract.**[TABLE:12].**The other less frequently isolated organisms were *Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis.*

In this study, 9 (12.16%) patients had one episode of bacteremia and the leading cause of gram positive bacteremia was *Staphylococcus aureus* and gram negative bacteremia was *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.**[TABLE:13]**. Chen *et al*[58]in their study documented bacteremia in 17% of SLE patients. The result of the present study is lower than the above study. *Salmonella* bacteremia was present in one patient. An increased association of Salmanellosis has been reported in SLE patients by Reyes *et al*. [75].

Pseudomonas aeruginosa was the common isolate from skin and soft tissue followed by *Staphylococcus aureus* and *Candida albicans***[TABLE:14]**.The other less frequently involved sites are ear, oral cavity and peritoneum **[TABLE:15]**.

Tuberculosis is an important cause of morbidity and mortality in patients with SLE especially in endemic countries like India. In the present study, 8 patients had

history of tuberculosis. In a study done in South Indian SLE patients by Kosaraju *et al*[44], history of tuberculosis has been reported in 6 patients.

Fungus was isolated from 6 patients and accounted for 8.1 % (6 out of 74) of infections **[TABLE:10]**. *Candida* was the most common fungus isolated. *Candida albicans* was isolated from oral scrapings of two patients who presented with oral thrush, one patient with fungal infection of nail and one patient with Urinary tract infection (UTI). *Candida tropicalis* was isolated from one patient with UTI. This is in agreement with Ginzler *et al*.[21] who observed that *Candida* infection is the common fungal infection in patients with SLE most often presenting with oral thrush. In a study done by H-Al-Rayes *et al*.[26]*Candida albicans* is the most common fungus associated with infections in SLE patients.

Aspergillus flavus was isolated from a case of SLE with ear infection. Ruiz Irastorza *et al*[25] reported a case of *Aspergillus* infection in their study in 83 patients with SLE.

Urinary tract was the most common site involved in patients with LN accounting for 62.96% of infections in them.**[TABLE:16,17]**. This was followed by involvement of blood (14.81%), respiratory tract (11.11%), skin and soft tissue (7.4%) and peritoneum (3.7%).

In this study, all the gram negative bacteria isolated were sensitive to Imipenem**[TABLE:18,19]**and all Gram positive cocci were found sensitive to Vancomycin. **[TABLE:20,21]**.ESBL production was detected in 30 of the 54 (55.55 %)

gram negative bacteria isolated**[TABLE:22]**. Among the ten strains of *Staphylococcus aureus* eight were found to be MRSA**[TABLE:23]**. *Salmonella typhi* was found sensitive to all the antibiotics tested.

All the five *Candida* isolated were sensitive to Amphotericin B, Itraconazole, Fluconazole and Voriconazole. One *Aspergillus flavus* was also sensitive to Amphotericin B, Itraconazole and Voriconazole. **[TABLE:24]**.

ANA was positive in 108 (98.18%) patients in the current study. Malaviya *et al*[53] in their study reported ANA positivity in 98% of SLE patients. The present study is comparable to the above study. In ANA detection by indirect IF using HEp 2 slides as substrate, the common pattern of nuclear staining observed was homogenous seen in 56 (51.85%) patients**[TABLE:26]**, Kosaraju *et al*[44]in their study on auto antibody profile in South Indian SLE patients have also reported homogenous pattern in 55.5% of patients. The other ANA patterns identified in the present study were speckled 28 (25.93%) and rim 24 (22.22%). Anti-ds DNA was positive in 70% of patients in this study **[TABLE:27]**. Similar result was also seen in the study of 75 SLE patients by Binoy *et al*. [69]in which about 76% of patients had positive Anti-ds DNA. 77 (70%) SLE patients were positive for both ANA and Anti-ds DNA.

In the present study low C3 levels were seen in 71 (64.5%) patients and low C4 levels were observed in 61 (55%) patients. This result is in consistent with the study of H-Al Rayes[26] in which C3 levels were decreased 65% of patients and C4 levels were decreased in 56% of patients. Both C3 and C4 levels were decreased in 60 (54.55%) SLE

patients. Low serum complement levels occur due to consumption of complement proteins as a result of immune complexes formation and deposition in various tissues.

In the present study, dose of prednisolone ≥ 20 mg/day was associated with an increased risk of infection with a p value of < 0.05 which is statistically significant**[TABLE:28]**. The studies by Pryor *et al*[72], Noel *et al*[65], Bosch *et al*[8], and Gladman *et al*[22] have identified steroid intake as a risk factor for infection in SLE patients. The prednisolone dose had a facilitating effect on infection in patients with SLE by Ruiz- Irastorza *et al*[25]. The present study is comparable to the above mentioned studies.

In the current study, the risk of infection was higher in patients receiving cyclophosphamide with a p value <0.05 which is statistically significant**[TABLE:29]**. Pryor *et al*[72] reported a higher rate of infection in patients receiving cyclophosphamide and steroids. The present study is in line with the above study. In this study, eight patients were on treatment with MMF out of which four developed infection. No significant association was found between infection and MMF with a p value more than 0.05**[TABLE:30]**. In the study by Hu *et al*. [33]it was found that the frequency of infection in SLE patients treated with MMF was less compared with patients on Cyclophosphamide (CPM)

Out of 110 SLE patients, 3 patients died during the study period. All the three patients were female and were on immunosuppressive therapy. Pneumonia was the cause of death in two patients and sepsis in one patient. Thus in all 3 cases, infection was the

cause of death. This finding is supported by studies by Barnatsky *et al*. [6]and Doria *et al*. [17]who have also reported infection as the major cause of death in SLE patients.

From this study it is very clear that SLE patients are at high risk for infections due to various immunological dysfunctions and use of immunosuppressants. Therefore it is essential to evaluate all fevers in SLE patients, as the symptoms of SLE may mimic infection in many ways. Also the cautious use of steroids and other immunosuppressants is vital in controlling infections in SLE patients.

SUMMARY

- A total of 110 SLE patients were included in this study. Samples were collected from these patients and processed according to standard methods.
- The female:male ratio was 8:1. The median age of onset of SLE was 22 years. Duration of SLE ranged from 1 month to 19 years with a mean of 37 months.
- Fifteen (13.6%) patients were children aged below 16 years.
- The common clinical manifestations were arthritis (66.36%), fever (49.09%) and skin rash (44.55%)
- 40.91% of patients had Lupus Nephritis and Class IV LN (42.22%) was common.
- Infectious episodes occurred in 60 (54.55%) patients.
- Urinary tract was the most common site of infection followed by respiratory tract.
- Bacterial infections were common accounting for 93.24% of infections.
- Gram negative bacteria were the predominant organisms isolated.
- *Escherechia coli* was the common uropathogen isolated from urinary tract.
- *Klebsiella pneumoniae*(66.67%) was the most common isolate from respiratory tract.
- *Klebsiella pneumoniae* (22.22%) and *Pseudomonas aeruginosa* (22.22%) were the common isolates from blood.
- *Pseudomonas aeruginosa* (50%) was the most common isolate from skin and soft tissue.

- All the gram negative bacteria were sensitive to Imipenem.
- 55.55% of the gram negative bacteria were ESBL producers.
- Out of the ten strains of *Staphylococcus aureus,* eight were found to be MRSA.
- All the isolates of *Staphylococcus aureus* were sensitive to Vancomycin.
- All the five *Candida* isolates were sensitive to Amphotericin B, Itraconazole, Fluconazole and Voriconazole.
- *Aspergillus flavus*(1) was sensitive to Amphotericin B, Itraconazole and Voriconazole.
- ANA was positive in 98.18 % of patients and homogenous was the most common pattern seen in indirect immunofluorescence.
- Anti-ds DNA was positive in 70% of patients. 70% of SLE patients were positive for both ANA and Anti-ds DNA.
- C3 and C4 levels were decreased in 63.64% and 55.45% of patients respectively. Both C3 and C4 levels were decreased in 54.55% of patients.
- Prednisolone dose ≥ 20mg per day was associated with increased risk of infection. Also patients on Cyclophosphamide and Prednisolone had an increased risk of infection. No association was found between MMF and infection.
- Out of 110 SLE patients, three patients died during the study period and infection was the major cause of death in all of them.

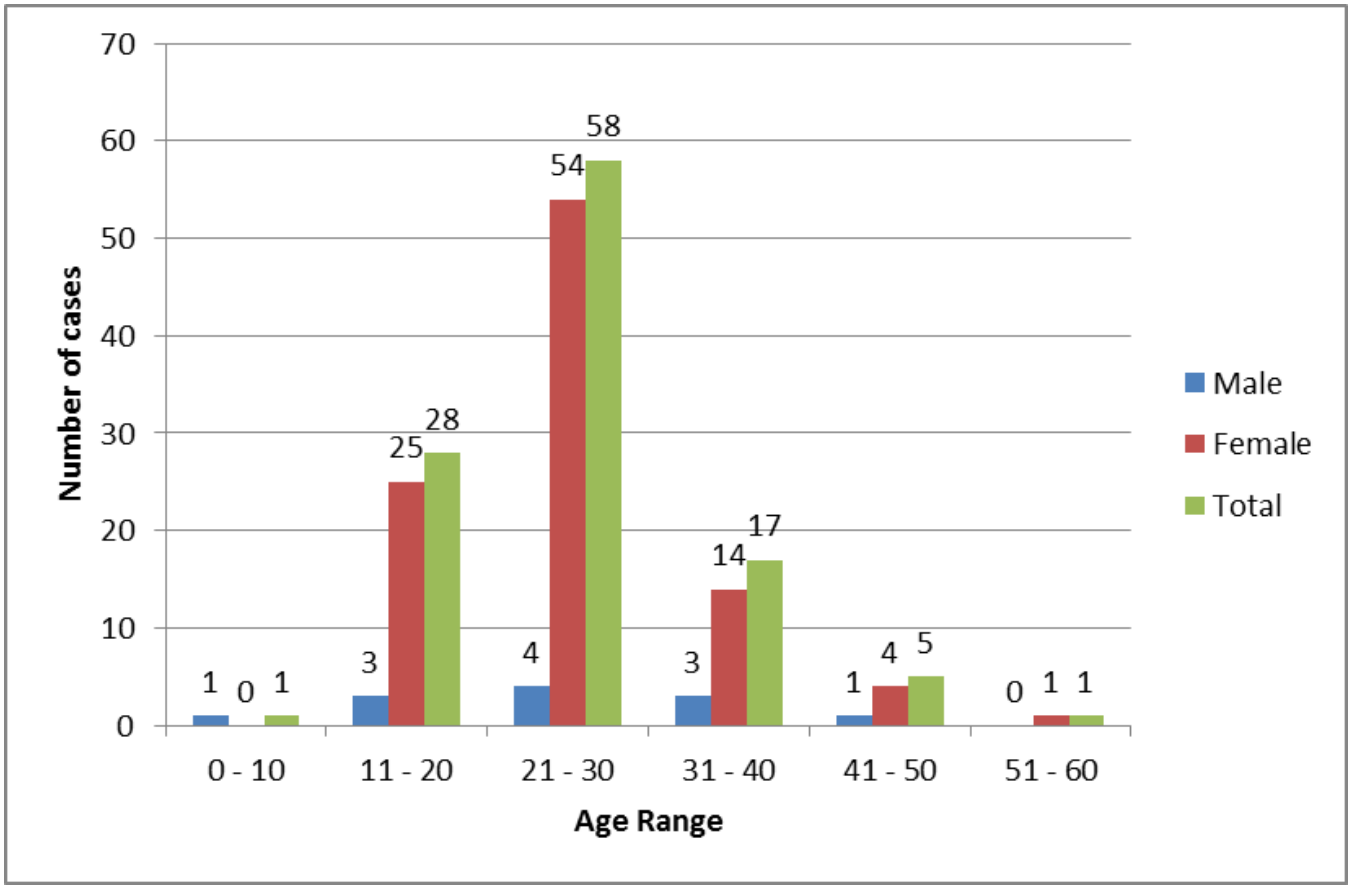
CONCLUSION

The present study was done to identify the bacterial and fungal infections in patients with SLE, which is the most common autoimmune disease involving multiple organ systems. Among the patients with SLE, arthritis was the most common manifestation followed by fever and skin rash. Infections were present in 54.55% of SLE patients. It was found that bacterial infections were common than fungal infections and urinary tract was the most common site of infection. *Escherechia coli* was the most common bacteria isolated and *Candida* was the common fungus isolated. Study of antimicrobial susceptibility of the isolates showed resistance to wide range of antibiotics. Among the 53 Gram negative bacteria isolated, 30 were ESBL producers. Out of the 10 *Staphylococcus aureus* isolated, 8 were found to be MRSA. In this study statistically significant association was found between infection and Prednisolone dose ≥ 20 mg/day and Prednisolone + cyclophosphamide.

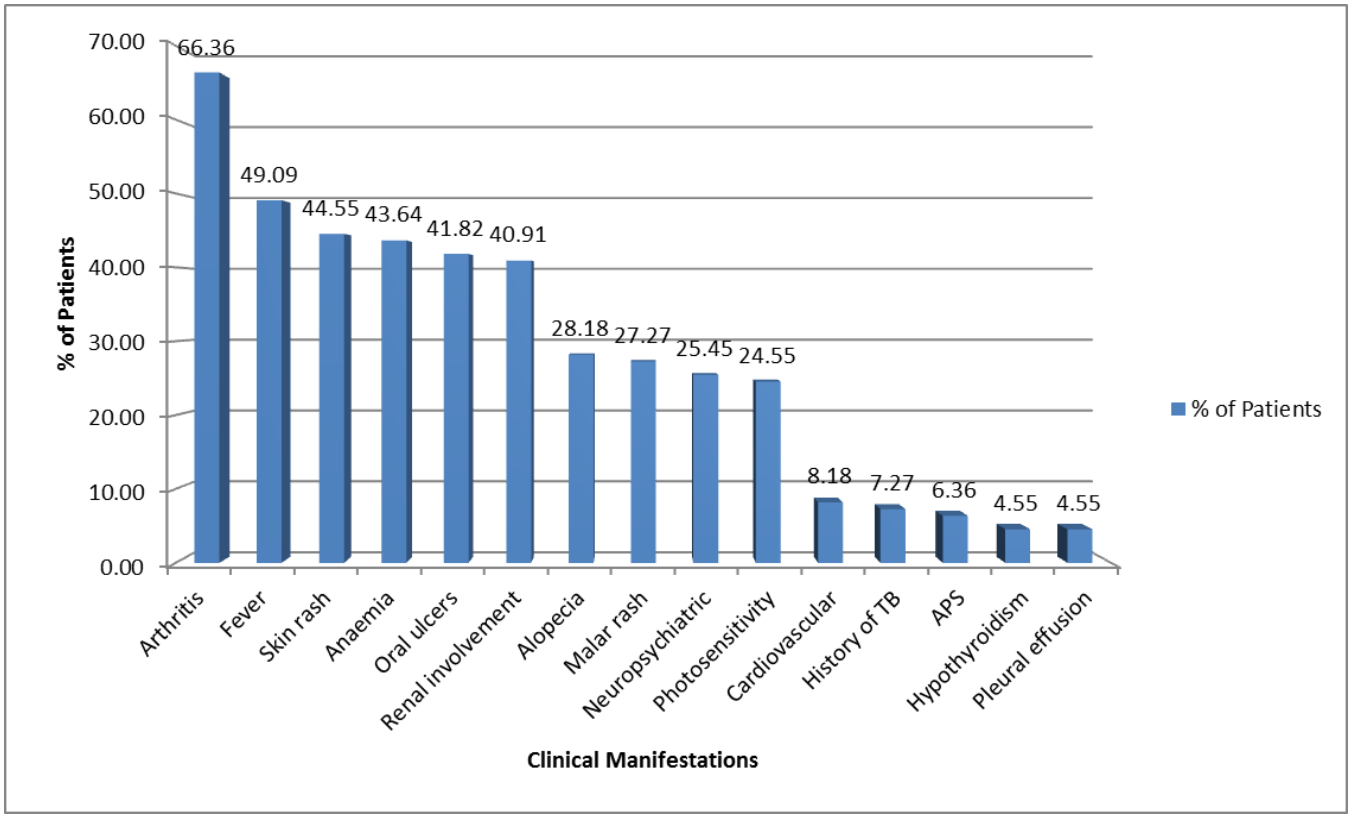
ANA was positive in 108 SLE patients and analysis of ANA patterns showed homogenous pattern to be the most common pattern. 77 patients were positive for both ANA and Anti-ds DNA. C3, C4 levels were found to be decreased in 63.64%, 55.45% of patients respectively. Both C3 and C4 levels were decreased in 60 SLE patients (54.55%).

There are only a few published articles on infections in SLE from south India. Hence the present study shows that there is correlation between high dose steroids and infection in SLE patients. This study also emphasizes the need for evaluation of all fevers and judicious use of steroids and other immunosuppressants in SLE patients.

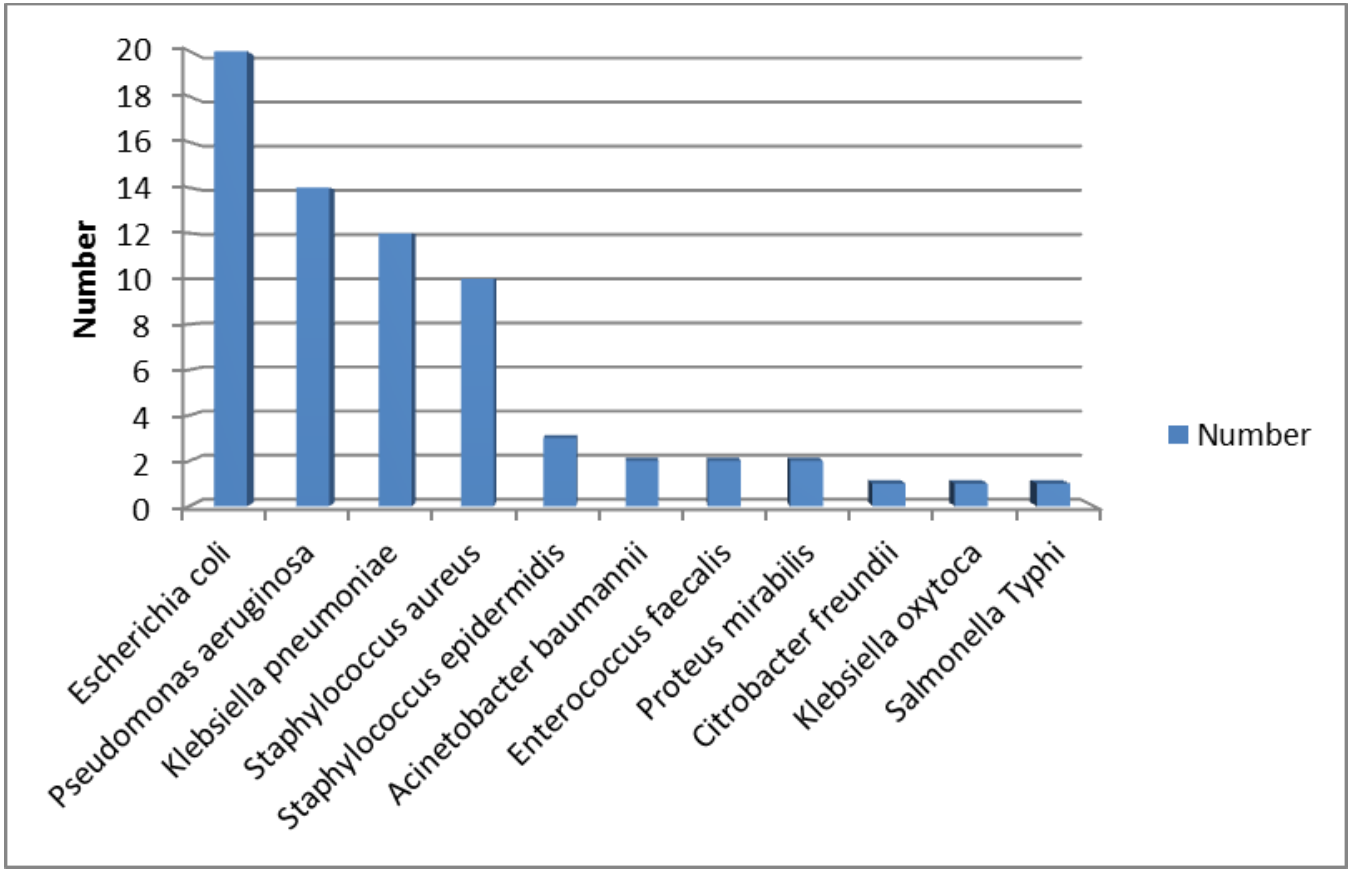
AGE AND SEX DISTRIBUTION OF SLE PATIENTS



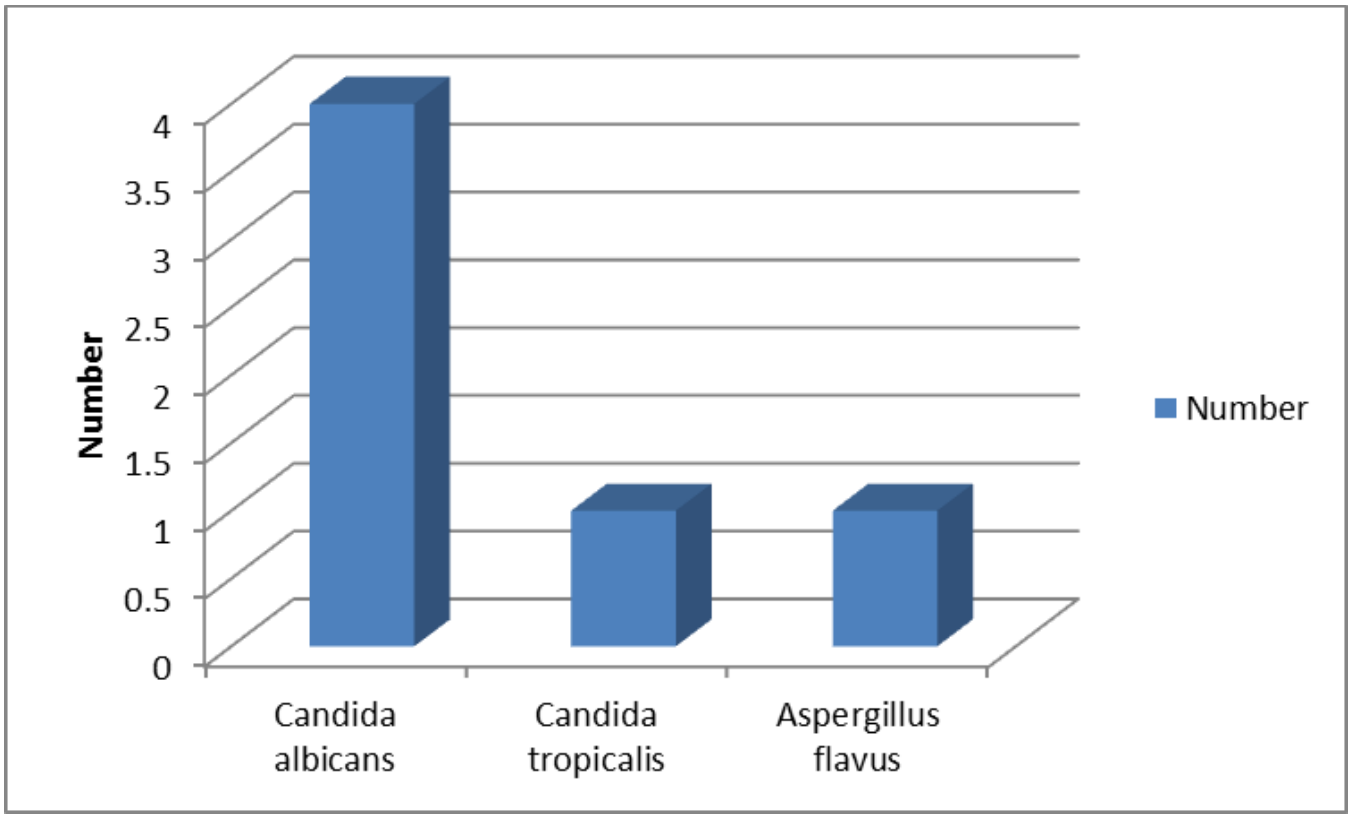
FREQUENCY OF CLINICAL MANIFESTATIONS IN SLE PATIENTS



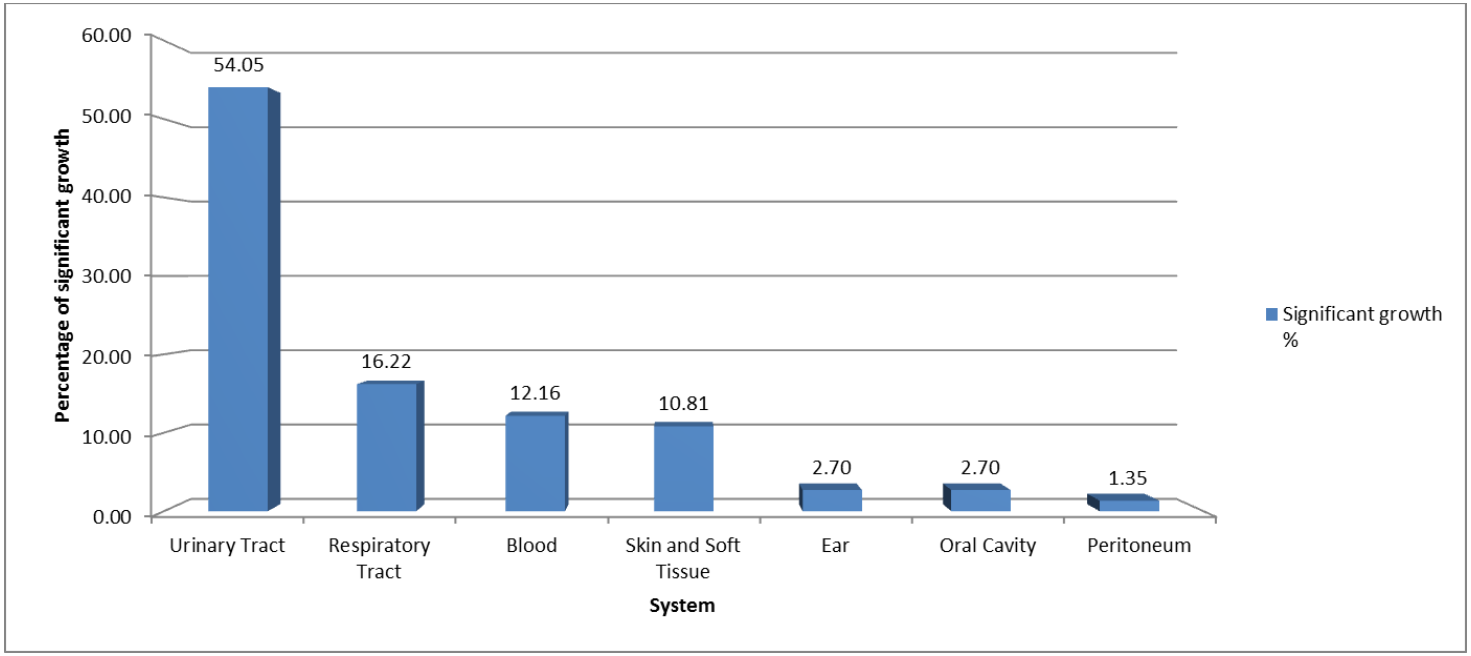
DISTRIBUTION OF BACTERIAL ISOLATES FROM ALL SITES



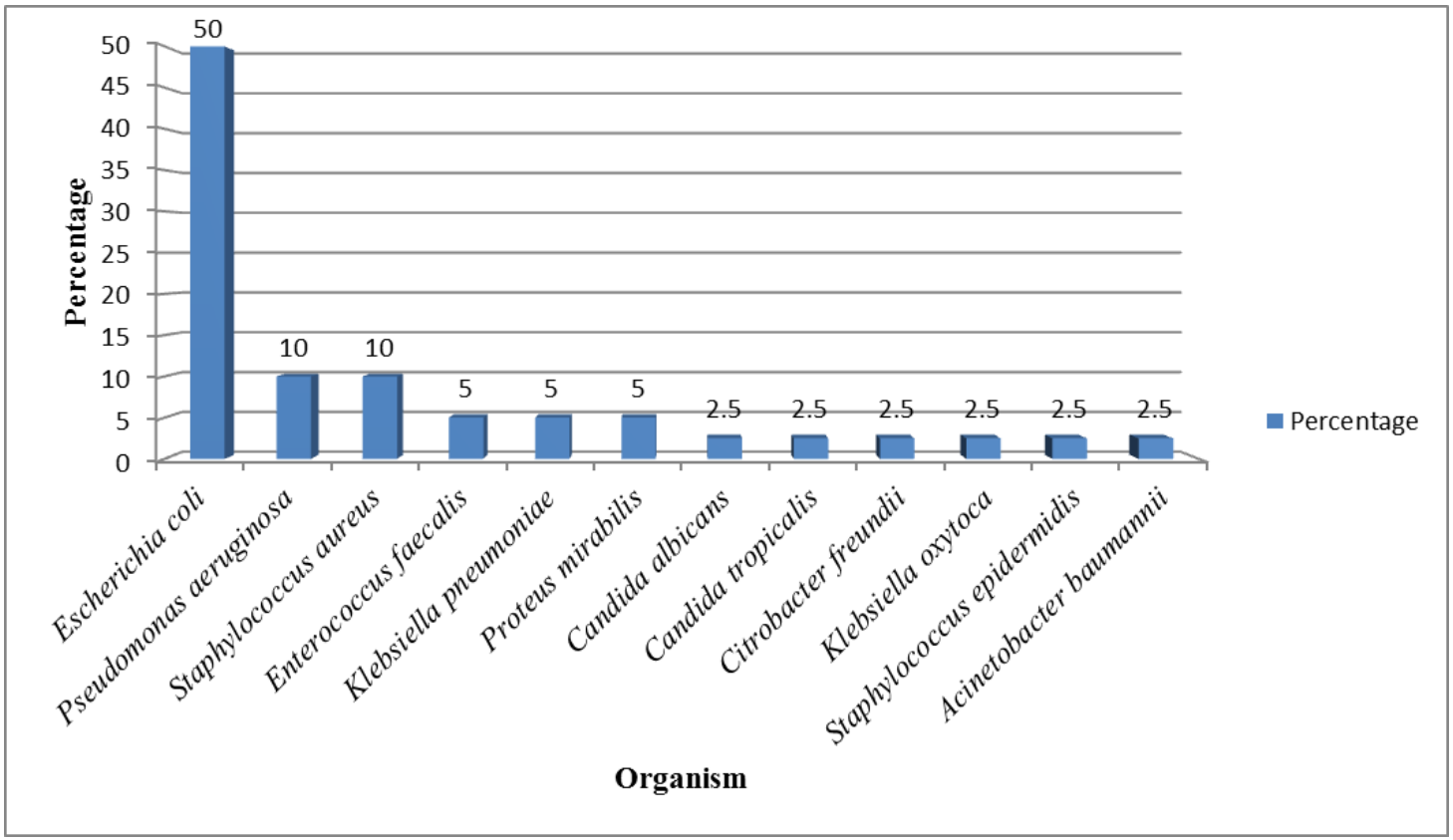
DISTRIBUTION OF FUNGAL ISOLATES FROM ALL SITES



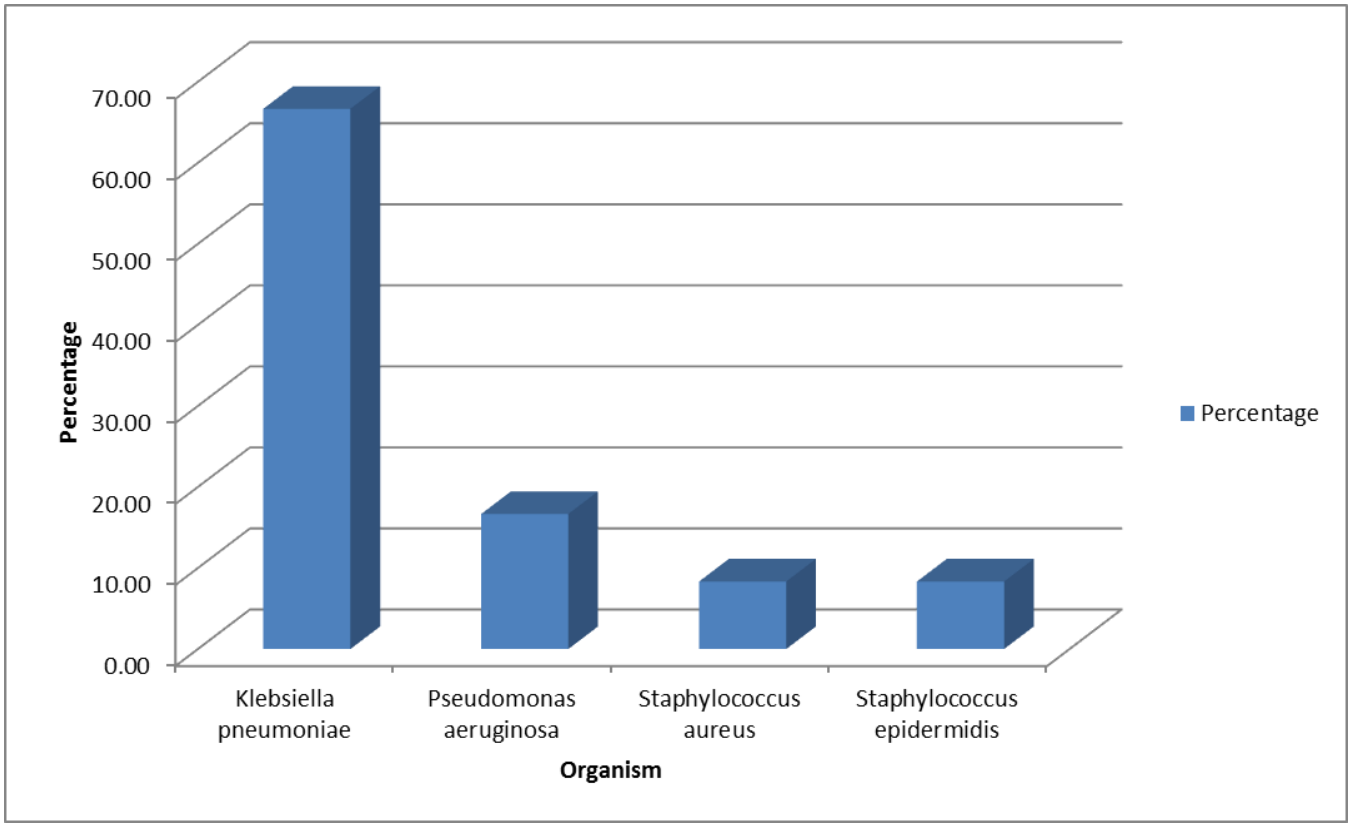
DISTRIBUTION OF INFECTIONS IN VARIOUS SITES



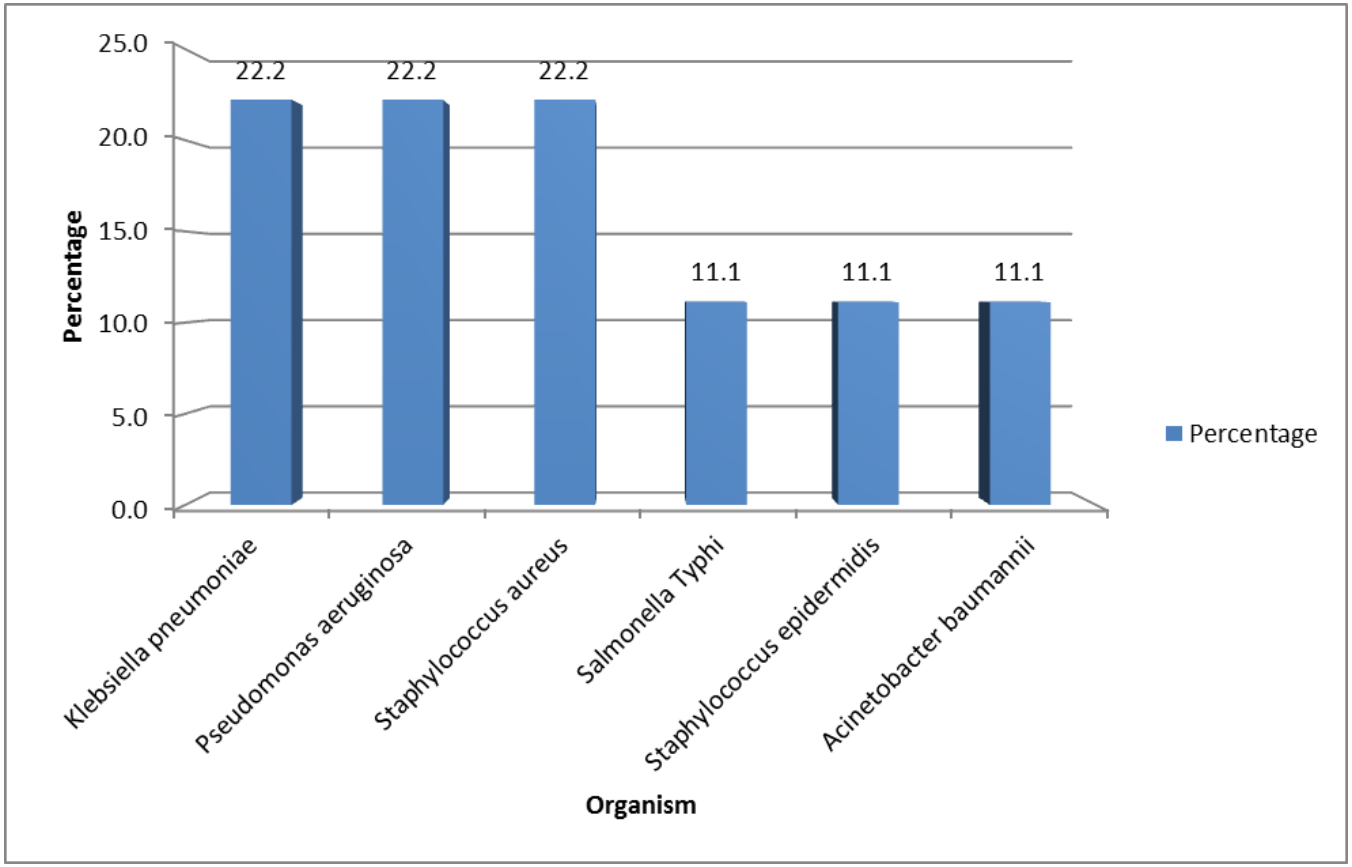
DISTRIBUTION OF ORGANISMS ISOLATED FROM URINARY TRACT



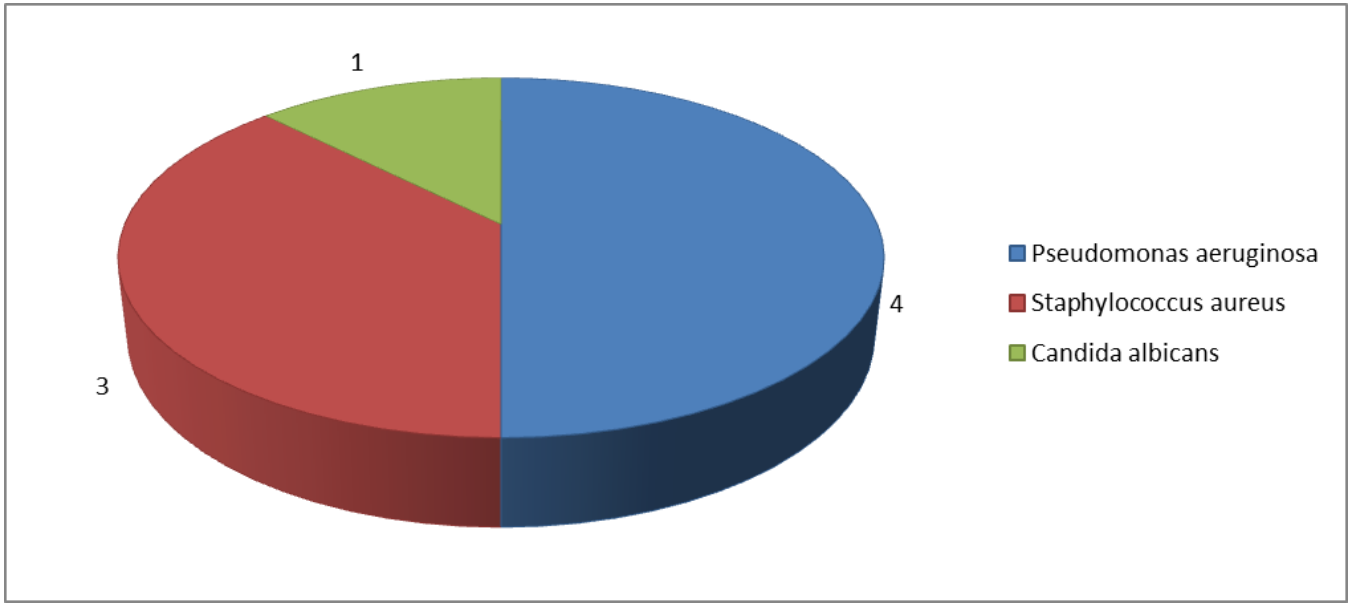
DISTRIBUTION OF ORGANISMS ISOLATED FROM RESPIRATORY TRACT



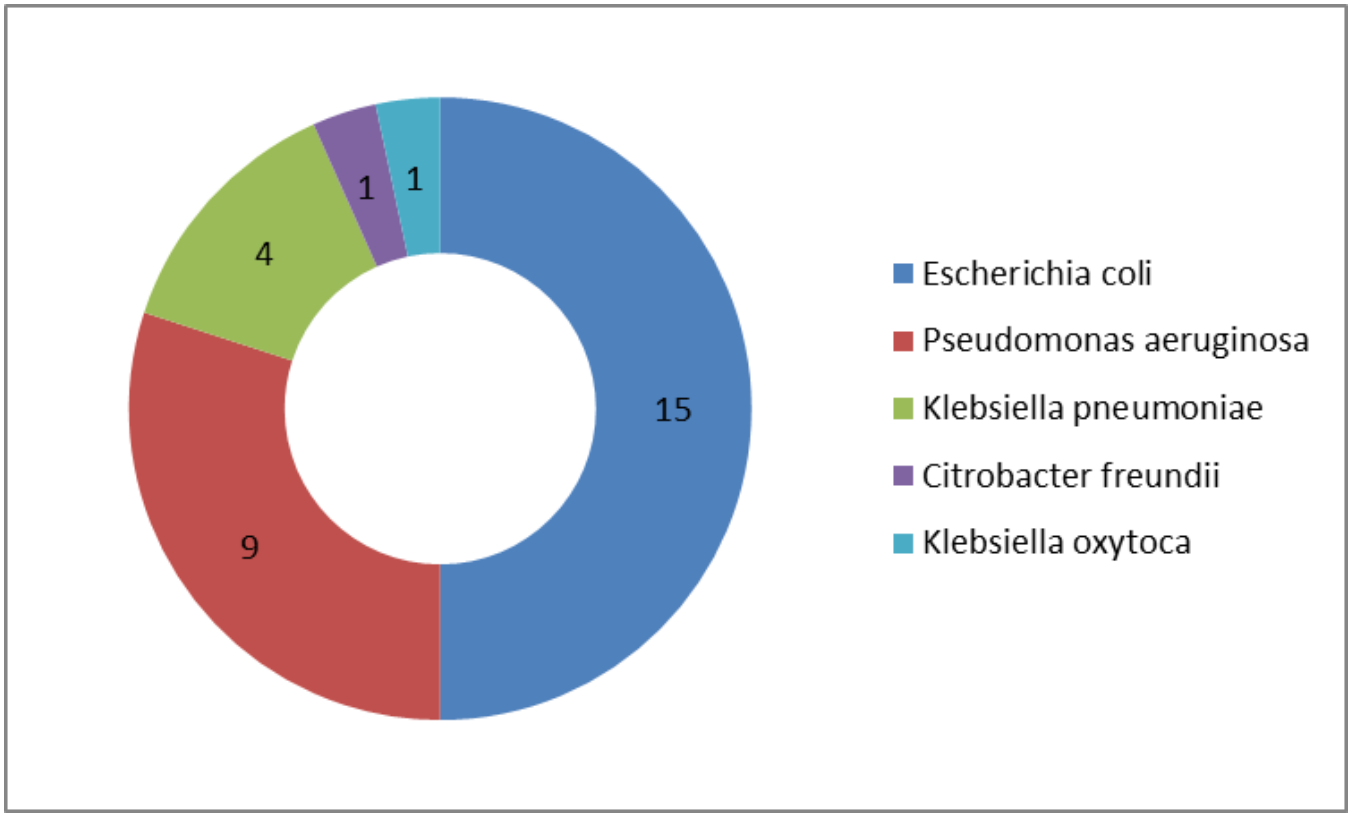
DISTRIBUTION OF ORGANISMS ISOLATED FROM BLOOD



DISTRIBUTION OF ORGANISMS ISOLATED FROM SKIN AND SOFT TISSUE



DISTRIBUTION OF ESBL PRODUCING ORGANISMS

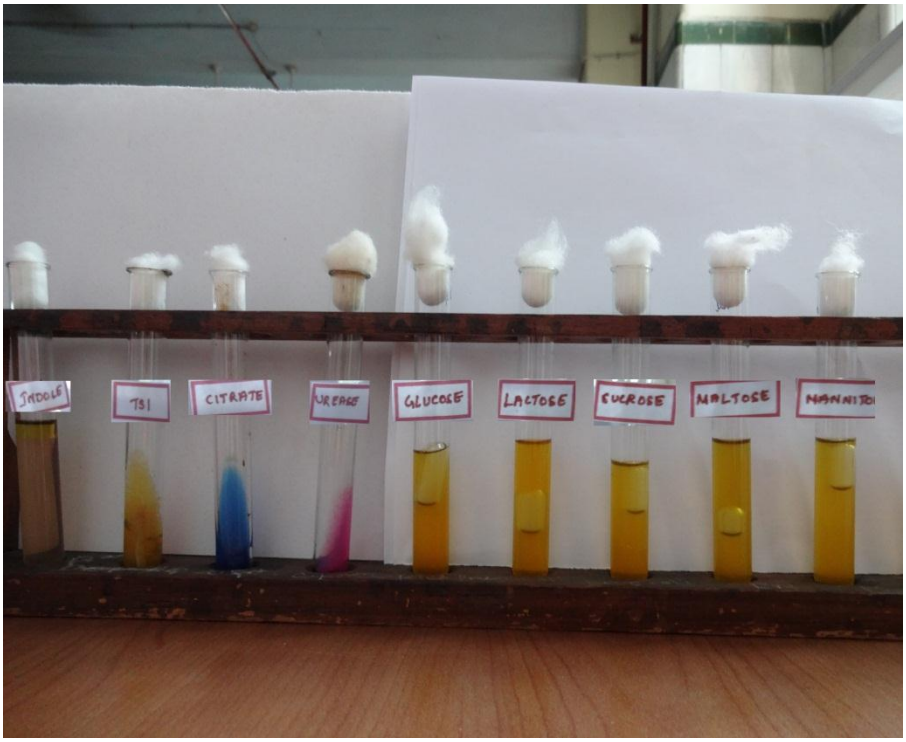


ESCHERECHIA COLI ON MACCONKEY AGAR

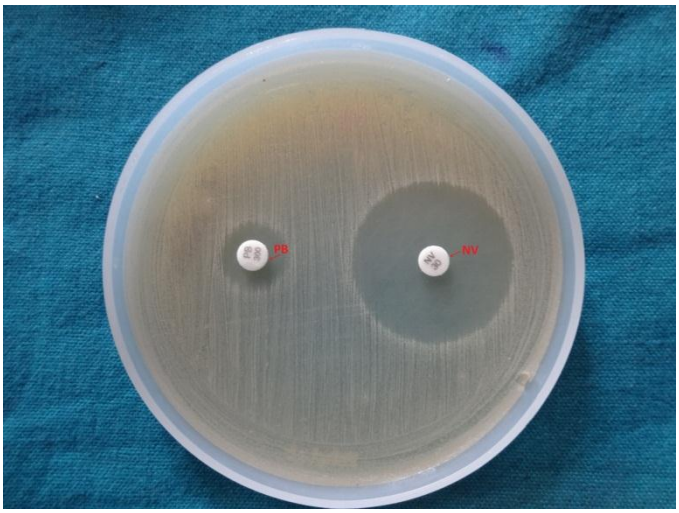
BIOCHEMICAL REACTIONS OF *ESCHERECHIA COLI*

KLEBSIELLA PNEUMONIAE ON MACCONKEY AGAR

KLEBSIELLA PNEUMONIAE – BIOCHEMICAL REACTIONS



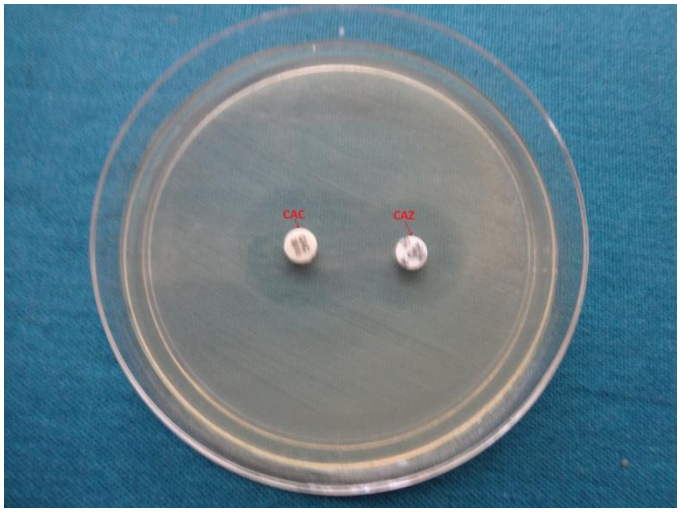
STAPHYLOCOCCUS EPIDERMIDIS – NOVOBIOCIN SENSITIVE, POLYMYXIN RESISTANT



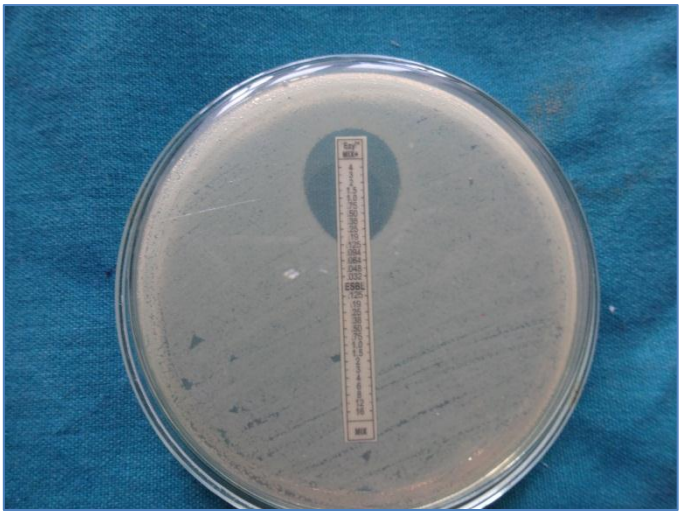
STAPHYLOCOCCUS EPIDERMIDIS – BIOCHEMICAL REACTIONS

ESBL DETECTION – *ESCHERECHIA COLI*– DDST

ESBL DETECTION – *ESCHERECHIA COLI*–PCT

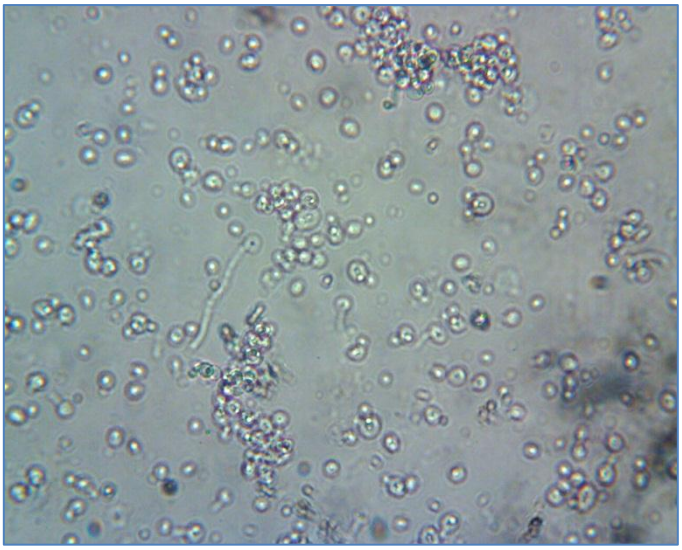


E-STRIP-ESBL DETECTION FOR *ESCHERECHIA COLI*



MIC OF VANCOMYCIN FOR *STAPHYLOCOCCUS AUREUS* (MIC – 1)

POSITIVE GERM TUBE – CANDIDA ALBICANS



CHROMagar CANDIDA

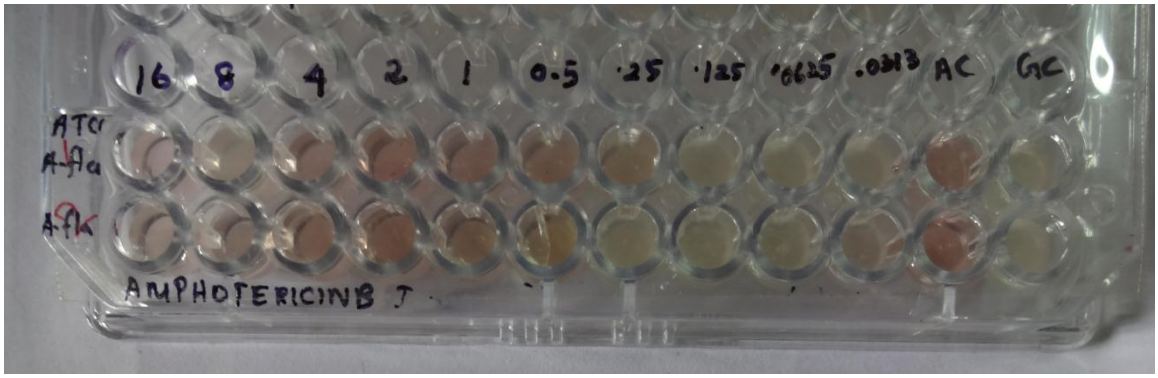
(a), (c), (d) – Candida albicans

(b) – Candida tropicalis

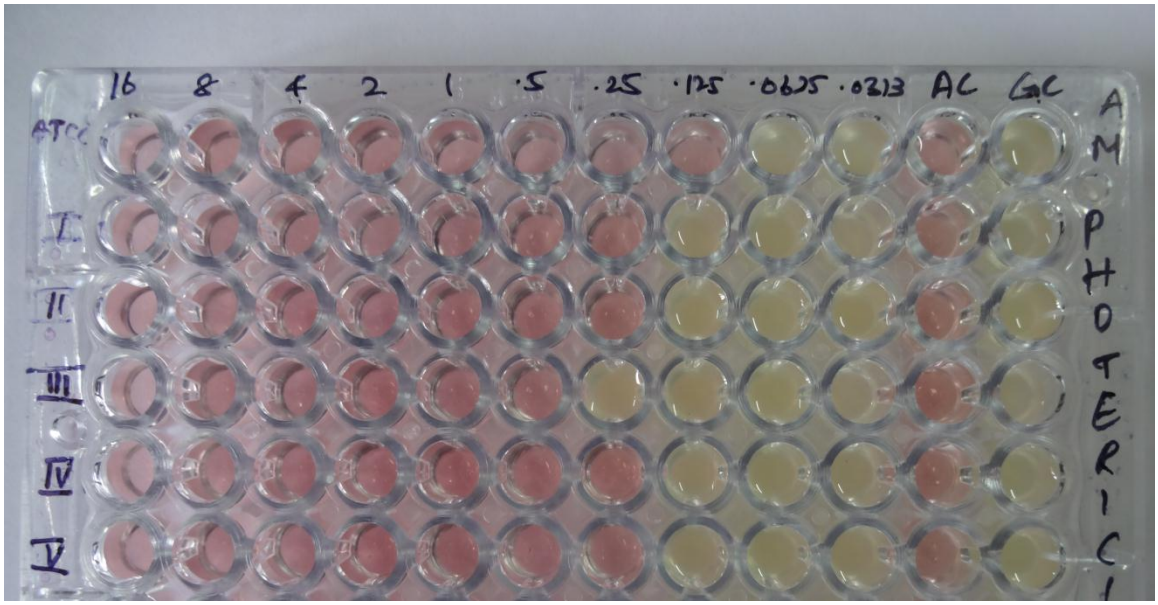
CANDIDA ALBICANS – SUGAR FERMENTATION

CANDIDA TROPICALIS – SUGAR FERMENTATION

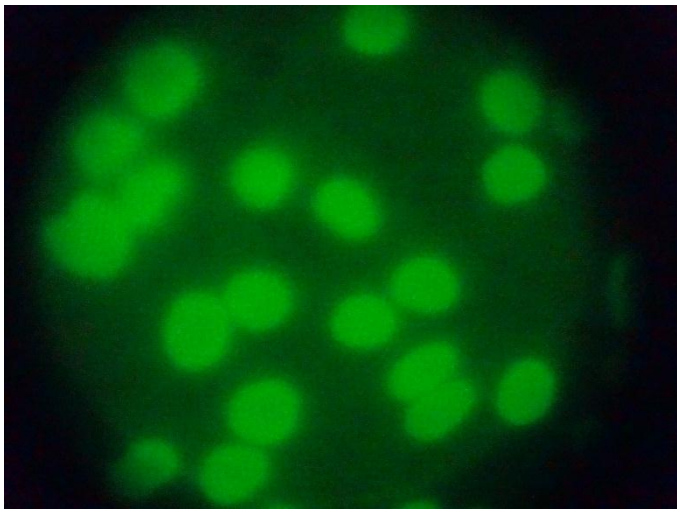
ANTIFUNGAL SUSCEPTIBILITY OF ASPERGILLUS FLAVUS BY MICROBROTH DILUTION METHOD (Sensitive ≤ 2 µg/ml)



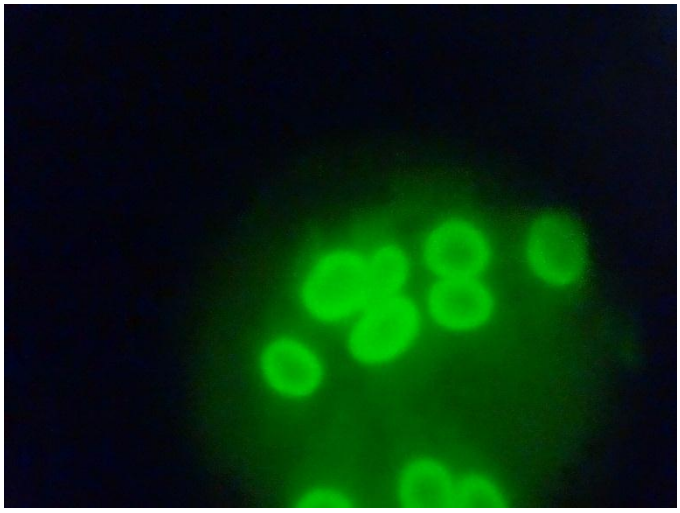
ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA BY MICROBROTH DILUTION METHOD (Sensitive ≤ 1 µg/ml)



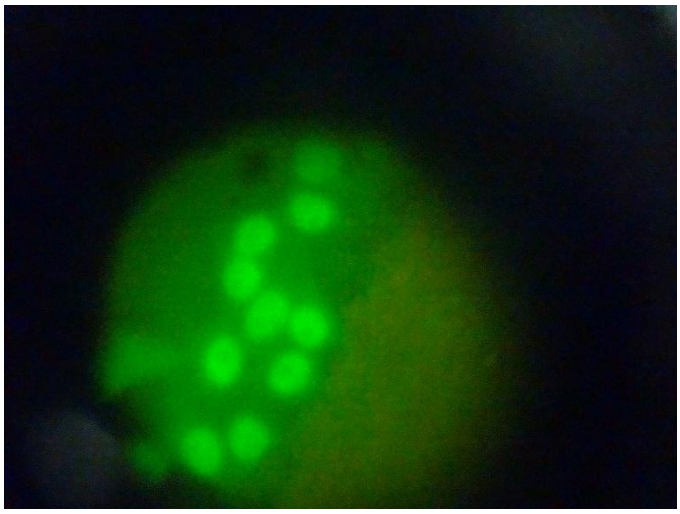
ANA BY IMMUNOFLOURESCENCE – HOMOGENOUS PATTERN USING HEp2 CELL



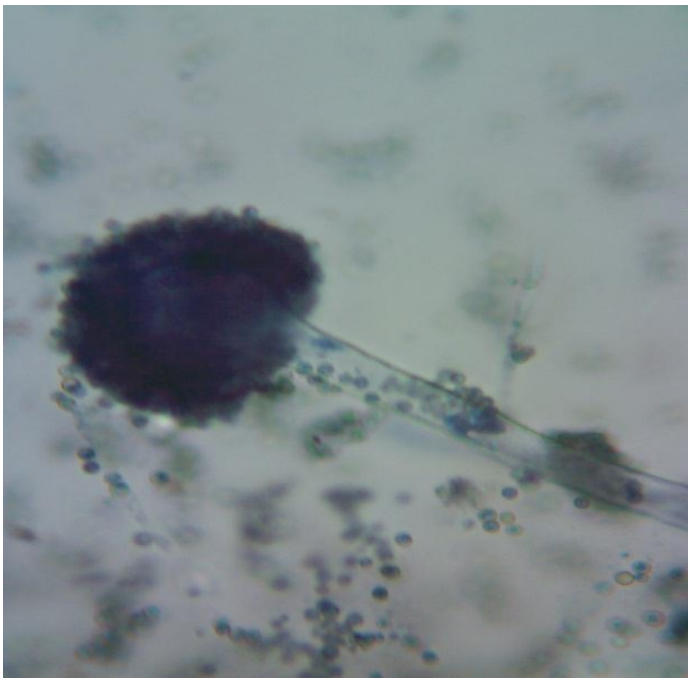
ANA BY IMMUNOFLOURESCENCE – RIM PATTERNUSING HEp2 CELL



ANA BY IMMUNOFLOURESCENCE – SPECKLED PATTERNUSING HEp2 CELL



ASPERGILLUS FLAVUS – LPCB MOUNT



PROFORMA

Name: OP / IP No:

Age: Ward:

Sex:

Occupation:

Address:

Presenting complaints:

Past Medical history:

Treatment history:

Associated Illness:

CLINICAL MANIFESTATIONS

1. Malar Rash
2. Discoid Rash
3. Photosensitivity
4. Oral ulcers
5. Arthritis
6. Serositis
7. Renal disorder
    a. Persistent Protenuria
    b. Cellular casts
8. Neurologic disorder
    a. Headache
    b. Seizures
    c. Psychisis

9. Haemotologic disorder
10. Immunologic disorder
11. Fever
12. Skin rashes
13. Alopecia

LABORATORY EVALUATION

1. Haemoglobin
2. Total count
3. Differential count
4. ESR
5. Blood sugar: Fasting, Postprandial
6. Urea
7. Creatinine
8. Urine routine examination
9. RF
10. ANA
11. dsDNA
12. C3 / C4 Levels

MICROBIOLOGICAL INVESTIGATION

a. Direct examination
    - Gram stain
    - Wet mount
    - KOH mount
    - India Ink preparation
b. Culture
    - Bacterial culture
    - Fungal culture
c. Antimicrobial susceptibility pattern

APPENDIX

A. STAINS AND REAGENTS

I. Gram staining

Methyl violet (2%)	l0g Methyl violet in l00ml absolute alcohol in 1litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolorizing agent
Carbol fuchsin 1%	Secondary stain

II. Lactophenol cotton blue stain

Lactic acid	20 ml
Phenol	20ml
Cotton blue (dye)	0.5g
Glycerol	40ml
Distilled water	20ml

III. 10% KOH

Potassium hydroxide	l0g
Glycerol	l0ml
Distilled water	80ml

IV. India Ink Stain

India Ink	150 ml
Merthiolate (1:1000)	3 ml
Tween 80 (1:10,000)	0.1 ml

Mixed, filtered and kept in bottles.

V. Ziehl-Neelsen stain

- Ziehl-Neelsen Carbol fuchsin

Basic fuchsin (powder)	5 g
Phenol (crystalline)	25 g
Absolute alcohol (ethanol)	50 ml
Distilled water	500 ml

- Sulphuric Acid 20 %
- Loeffler's methylene blue 0.5 %

B. MEDIA USED

1. MacConkey agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1 ltr
Agar	20g

2% neutral red in 50% ethanol	3.5ml
10% lactose solution	100 ml

Dissolve peptone and taurocholate in water by heating. Add agar anddissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake welland mix.Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15minutes.

2. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5 g
Distilled water	1 Liter
Agar	10 g

Dissolve ingredients in distilled water by boiling, and add 5% sheepblood(sterile) at 55°C adjust pH to 7.4.

3. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added withconstant agitation. After addition of blood, kept in water bath and heating was continued till the

blood changed tochocolate color. Cooled to about 50° C andpoured about 15ml into petri dishes with sterile precaution.

4. Sabouraud's dextrose agar

Dextrose	40g
Peptone	l0g
Agar	20g
Distilled water	l000ml

pH = 5.6

5. Mueller – Hinton Agar

Beef infusion	300ml
Caeseinhydrolysate	17.5g
Starch	1.5g
Agar	10g
Distilled water	1ltr

pH = 7.4. Sterilise by autoclaving at 121ºC for 20 minutes.

6. Cystine lactose electrolyte – deficient medium

Peptone	4 g
Tryptone	4 g

Meat extract powder	3 g
Lactose	10 g
L- cystine	0.128 g
Bromothymol blue	0.02 g
Agar	15 g
Water	1 L

Sterilize by autoclaving at 121ºC for 20 minutes.

7. Lowenstein Jensen Medium

Mineral salt solution	600 ml
Malachite green solution	20 ml
Beaten egg	

After mixing the complete medium distribute in 5 ml amounts in sterile screw capped bottles. Lay the bottles on their sides and inspissate at 80^0C – 85^0C for 3 consecutive days.

8. Xylose lysine deoxycholate agar

Yeast extract	3.0 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g

l-Lysine HCL	5.0 g
Sodium chloride	5.0 g
Sodium deoxycholate	2.5 g
Sodium thiosulphate	6.8 g
Ferric ammonium citrate	0.8 g
Phenol red	0.08 g
Agar	15 g
Water	1 L

pH 7.4. Sterilize by autoclaving at 121ºC for 20 minutes.

9. Selenite F broth

Sodium hydrogen selenite	4 g
Peptone	5 g
Lactose	4 g
Disodium hydrogen phosphate	9.5 g
Sodium dihydrogen phosphate	0.5 g
Sterile water	1 L

Steam for 20 min at 100 ºC

10. BHI broth

Brain infusion broth	250 ml
Heart infusion broth	750 ml
Sodium polyanetholsulphonate	0.25 g

pH: 7.4 to 7.6. Sterilize by autoclaving at 121ºC for 20 minutes

C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1. Oxidase Reagent

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2. Catalase Reagent

3% hydrogen peroxide

3. Indole test

Kovac's reagent contains the following:

Amyl or isoamyl alcohol	150ml
Para dimethyl amino benzaldehyde	10g
Concentrated hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare insmall quantities and store in the refrigerator. Shake gently before use.

4. **Christensen's Urease test medium**

Peptone	1g
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	l0ml
Sterile 20% urea solution	l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basalmedium without glucose and urea, adjust to pH 6.8-6.9 and sterilize byautoclaving in a flask at 121°C for 30 minutes. Cool to about 50°C, add the glucose and urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20g
Bromothymol blue 0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes.

6. Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	1g
Lactose	l0g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
Sodium thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	l2ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at121°C for 15 minutes and cool to form slopes with deep butts.

7. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr

Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter, dispense in 5mlamounts and sterilize at 121°C for 15min. Sterilize the glucosesolution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	10mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A:

Alpha naphthol	5g
Ethyl alcohol	100ml

Reagent B:

Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium

To the basal medium of peptone water, add sterilized1% sugars, bromothymol blue indicator with Durham's tube.

Basal medium: Peptone water

Sugar solutions:

Sugar	1ml
Distilled water	l00ml

pH = 7.6.

9. Mannitol motility medium

Agar	5g
Peptone	1g
Potassium nitrate	1 g
Mannitol	2 g
Phenol red indicator	
Distilled water	l000ml

pH 7.2

10. Phenolphthalein diphosphate agar

- ✓ Sterilize a 1% aqueous solution of sodium phenolphthalein diphosphateby filtration and store at 4ºC
- ✓ Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50ºCand pour plates
- ✓ Grow the staphylococcus overnight at 37ºC on the medium
- ✓ Invert the plate and pour a few drops of ammonia solution SG 0.88 intothe lid

- ✓ Read as positive a culture whose colonies turn bright pink within a fewminutes.The color soon fades.

11. Potassium nitrate broth

Potassium nitrate (KN03)	0.2g
Peptone	5.0g
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 mlamount and autoclaved.

12. Phenyl alanine deaminase test

Yeast Extract	3g
DL-Phenylalanine	2 g
Disodium hydrogen phosphate	l g
Sodium Chloride	5 g
Agar	12 g
Distilled water	1 lr

pH 7.4

Distributed in tubes and sterilized by autoclaving at 121°C for 15minutes, allowed to solidify as long slopes.

13. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml

Sugar to be tested	20g
Water	1liter

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the color of thesolution yellow.Dissolve the peptone and Andrade's indicator in 1liter of water and add20g of the sugar; sugars to be tested generally include glucose, sucrose, lactoseand maltose. Distribute 3ml amounts in standard test tubes containing aninverted Durham tube. Sterilize by steaming at 100^0C for 30 minutes on 3consecutive days.

ABBREVIATIONS

ANA	-	Antunuclear Antibody
aPL	-	Antiphospholipid Antibody
ATCC	-	American Type Culture Collection
BAL	-	Bronchoalveolar lavage
CIE	-	Counter Immunoelectrophoresis
CLSI	-	Clinical and Laboratory Standards Institute
CPM	-	Cyclophosphamide
DDST	-	Double disk diffusion synergy test
ELISA	-	Enzyme linked Immunosorbent Assay
ESBL	-	Extended Spectrum Beta Lactamase
FANA	-	Indirect immunofluorescence Antinuclear antibody test
HLA	-	Human Leukocyte antigen
ID	-	Immunodiffusion
LN	-	Lupus Nephritis
MHC	-	Major Histocompatibility Complex
MIC	-	Minimum Inhibitory Concentration
MMF	-	Mycophenolate mofetil

MRSA	-	Methicillin resistant Staphylococcus aureus
NSAID	-	Nonsteroidal antiinflammatory drugs
PCT	-	Phenotypic confirmation test
PBS	-	Phosphate buffered saline
RIA	-	Radioimmunoassay
RID	-	Radial immunodiffusion
SLE	-	Systemic lupus erythematosus
UTI	-	Urinary tract infection

BIBLIOGRAPHY

1. Abramson S, Kramer SB, RadinA *et al*. Salmonella bacteremia in systemic lupus erythematosus. Eight year experience at a municipal hospital.*Arthritis Rheum* 1985; 28: 75–79.

2. Abu-Shakra M, Urowitz MB, Gladman DD, *et al*.: Mortality studies in systemic lupus erythematosus: results from a single center. I. Causes of death. *J Rheumatol* 1995, 22:1259–1264.

3. American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes.*Arthritis Rheum* 42:599-608, 1999.

4. Archana R. Vasudevan, Ellen M. Ginzler. Rheumatology, Fifth edition, *Volume* 2, Chapter 123, Clinical features of Systemic lupus erythematosus, pg. 1229-46.

5. Arnett FC, Edworthy SM, Bloch DA *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315–324.

6. Barnatsky S, Boivin JF, Joseph L *et al*. Mortality in systemic lupus erythematosus. *Arthritis Rheum* 2006; 54: 2550–57.

7. Bellomio V, Spindler A, Lucero E, *et al*.: Systemic lupus erythematosus: mortality and survival in Argentina: a multicenter study. *Lupus* 2000, 9:377–381.

8. Bosch X, Guilabert A, Pallares L, Cervera R, Ramos-Casals M, Bové A, Ingelmo M, Font J: Infections in systemic lupus erythematosus: a prospective and controlled study of 110 patients. *Lupus* 2006, 15:584-589.

9. Boumpas DT, Sidiropoulos P, Bertsias G: Optimum therapeutic approaches for lupus nephritis: What therapy and for whom? *Nat ClinPractRheumatol* 1:22-30, 2005.

10. Breban M, Meyer O, Bourgeois P, *et al.:* The actual survival rate in systemic lupus erythematosus: study of a 1976 cohort. *ClinRheumatol* 1991, 10:283–288.

11. C.C.Mok, K.W.Lee, C.T.K.Ho, C.S.Lau, R.W.S.Wong.A prospective study of survival and prognostic indicators of systemic lupus erythematosus in a southern Chinese population.*Rheumatology* 2000; 39: 399-406.

12. Clinical and Laboratory Standards for Antimicrobial Susceptibility Testing; Twenty first Informational Supplement M100 – S21. January 2011.

13. Daniel J. Wallace, BevraHannahs Hahn. Dubois' Lupus Erythematosus, 7th edition, Chapter 45, Infections in Systemic Lupus Erythematosus, pg. 901-910.

14. de Luis A, Pigrau C, Pahissa A *et al*. Infections in 96 cases of systemic lupus erythematosus. *Med Clin (Barc*) 1990; 94: 607–610.

15. De Marcaida JA, ReikJr L: Disorders that mimic central nervous system infections. *NeurolClin* 17:901-941, 1999.

16. Deapen D, Escalante A, Weinrib L, *et al.* A revised estimate of twin concordance in systemic lupus erythematosus.*Arthritis Rheum* 1992; 35:311.

17. Doria A, Iaccarino L, Ghirardello A *et al*. Long-term prognosis and causes of death in systemic lupus erythematosus. *Am J Med* 2006; 119: 700–706.

18. Faller G, Thomson PD, Kala UK, Hahn D. Demographics and presenting clinical features of childhood systemic lupus erythematosus. *S Afr Med* J 2005; 95: 424–427.

19. Feinglass EJ, Arnett FC, Dorsch CA, *et al.*: Neuropsychiatric manifestations of systemic lupus erythematosus: diagnosis, clinical spectrum, and relation to other features of the disease. *Medicine* 1976, 55:323–339.

20. Gary S. Firestein, Ralph C. Budd, Edward D. Harris, Jr., Iain B. McInnis, Schaun Ruddy, John S. Sergent. Kelley's textbook of Rheumatology, 8th edition, *Vol*. II, Chapter 75, Clinical features and treatment of Systemic Lupus Erythematosus, pg. 1263-1300.

21. Ginzler E, Diamond H, Kaplan D *et al*. Computer analysis of factors influencing frequency of infection in systemic lupus erythematosus.*Arthritis Rheum* 1978; 21: 37–44.

22. Gladman DD, Hussain F, Ibañez D, Urowitz MB: The nature and outcome of infection in systemic lupus erythematosus. *Lupus* 2002, 11:234-239.

23. Gladman DD, Urowitz MB, ChaudhryAhluwalia V., *et al*: Predictive factors for symptomatic osteonecrosis in patients with systemic lupus erythematosus. *J Rheumatol* 28:761-765, 2001.

24. Grande JP, Balow JE: Renal biopsy in lupus nephritis. *Lupus* 7:611-617, 1998.

25. Guillermo Ruiz-Irastorza, Nerea Olivares, Ioana Ruiz-Arruza, Agustin Martinez-Berriotxoa, Maria-Victoria Egurbide and Ciriaco Aguirre. Predictors of major infections in systemic lupus erythematosus.*Arthritis Research & Therapy* 2009, 11:R109.

26. H Al-Rayes, R Al-Swailem, M Arfin, S Sobki, S Rizvi, M Tariq. Systemic lupus erythematosus and infections: a retrospective study in Saudis. *Lupus* 2007, 16: 755–763.

27. Halberg P, Alsbjorn B, Trolle-Balslov J, *et al*.: Systemic lupus erythematosus: follow-up study of 148 patients. I. Classification, clinical and laboratory findings, course and outcome. *ClinRheumatol* 1987, 6:13–21.

28. Hanly JG.Neuropsychiatric lupus.*Rheum Dis Clin North Am* 2005; 31: 273-298.

29. Hashimoto H, Tsuda H, Hirano T, *et al*.: Differences in clinical and immunologic findings of systemic lupus erythematosus related to age. *J Rheumatol* 1987, 14:497–501.

30. Hector Molina. Complement and immunity.*Rheumatic disease clinics of North America.* February 2004. Vol.30.

31. Hellmann DB, Petri M, Whiting-O' Keefe Q.Fatal infections in SLE: the role of opportunistic organisms. *Medicine* 1987;66: 341-348.

32. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.

33. Hu W, Liu Z, Chen H *et al*. Mycophenolatemofetilvs cyclophosphamide therapy for patients with diffuse proliferative lupus nephritis. *Chin Med J (Engl*) 2002; 115: 705–709.

34. Insoo Kang, Sung Park. Infectious complications in SLE after immunosuppressive therapies.*Current opinion in rheumatology*.Sep 2003; *Vol*.15.No.5.

35. Jacobsen S, Petersen J, Villman S *et al.* Mortality and causes of death of 513 Danish patients with SLE. *Scandinavian Journal of Rheumatology*; 1999.*Vol*.2 pg. 75-80.

36. Jacqueline Trager, Michael M. Ward. Mortality and cause of death in systemic lupus erythematosus.*Current opinion in rheumatology* 2001, 13: 345-351.

37. JagdishChander Textbook of Medical Mycology, 3rd Edition, Section V, Chapter 20, Candidiasis, pg. 266-283.

38. James JA, Kaufman KM, Farris AD, *et al*. An increased prevalence of Epstein-Barr Virus infections in young patients suggests a possible etiology for systemic lupus erythematosus. *J Clin Invest* 1997; 100: 3019.

39. Jeong SJ, Choi H, Lee HS, Han SH, Chin BS, Baek JH, Kim CO, Choi JY, Song YG, Kim JM: Incidence and risk factors of infection in a single cohort of 110 adults with systemic lupus erythematosus. *Scand J Infect Dis* 2009, 41:268-274.

40. JL Huang, KW Yeh, TC Yao, YL Huang, HT Chung, LS Ou, WI Lee, LC Chen. Pediatric lupus in Asia.*Lupus*Oct 2010: *vol*.19, no.12, 1414-1418.

41. John Cazin JR. A simplified routine method for determining carbohydrate fermentation by yeasts.*Mycopathologia*1969; *Vol*. 37: no. 4313-4319.

42. Jose C. Crispin, George C. Tsokos. Rheumatology, Fifth edition, *Volume* 2, Chapter 128, Pathogenesis of lupus, pg. 1289-94.

43. Julia F. Simard, Karen H. Costenbader. Rheumatology, Fifth edition, *Volume* 2, Chapter 122, Epidemiology and classification of Systemic lupus erythematosus, pg. 1223-28.

44. K Kosaraju, S Shenoy, U Suchithra.A cross-sectional hospital-based study of autoantibody profile and clinical manifestations of systemic lupus erythematosus in south Indian patients. *IJMM*,(2010) 28(3): 245-7.

45. Klemperer P, Pollack AD, Bachr G. Pathology of disseminated lupus erythematosus. *Arch Pathol* 1941; 32: 569-631.

46. Li EK, Cohen MG, Ho AK, *et al.:* Salmonella bacteremia occurring concurrently with the first presentation of systemic lupus erythematosus. *Br J Rheumatol* 1993, 32:66–67.

47. Lim E, Koh WH, Loh SF *et al.* Non-typhoidal salmonellosis in patients with systemic lupus erythematosus.A study of fifty patients and a review of the literature.*Lupus* 2001; 10: 87–92.

48. Longo, Fauci, Kasper, Hauser, Jameson, Loscalzo. Harrison's principles of Internal Medicine, *vol*. 2, chapter 319, Systemic Lupus Erythematosus, pg. 2724 - 35

49. Mackie & McCartney Practical Medical Microbiology, Fourteenth edition, Section D, Chapter 41, Fungi, pg. 695-717.

50. Madhavan R et al. Systemic Lupus Erythematosus: The Madras Experience. *J AssocPhys India* 1988; 36: 481-4.

51. Mahler M, Kessenbrock K, Raats J, *et al*. Characterization of the human autoimmune response to the major C-terminal epitope of the ribosomal P proteins. *J Mol Med* 2003; 81: 194-204.

52. Malaviya AN *et al*. Systemic lupus erythematosus in India.*Lupus*. 1997; 6(9):690-700.

53. Malaviya AN, Misra R, Banerjee S *et al*. Systemic Lupus Erythematosus in North Indian Asians: A prospective analysis of clinical and immunological features. *Rheumatology International* 1986; 6: 97-101.

54. Malaviya AN. (1988) Systemic lupus erythematosus in northern India: A review of 329 cases. *J Assoc Physicians India* 1988; 36: 476-480.

55. Malaviya AN. (1993) Prevalence of systemic lupus erythematosus in India.*Lupus*. 1993 Apr; 2(2):115-8.

56. Malaviya AN. (1997) Occasional series-Lupus round the world: systemic lupus Erythematosus in India. *Lupus* 1997; 6: 690-700.

57. Michet CJ, McKenna CH, Elveback LR, *et al*.: Epidemiology of systemic lupus erythematosus and other connective tissue diseases in Rochester, Minnesota,1950 through 1979. *Mayo ClinProc* 1985, 60:105–113.

58. MJ Chen, H M Tseng et al. Long term outcome and short term survival of patients with Systemic Lupus Erythematosus after bacteremia episodes: 6 year follow-up. *Rheumatology* (2008) 47(9):1352-57.

59. Mok CC, Lee KW, Ho CTK, *et al*.: A prospective study of survival and prognostic indicators of systemic lupus erythematosus in a southern Chinese population. *Rheumatology* 2000, 39:399–406.

60. Moroni G, Ventura D, Riva P, *et al*: Antiphospholipid antibodies are associated with an increased risk for chronic renal insufficiency in patients with lupus nephritis. *Am J Kidney Dis* 43:28-36, 2004.

61. Moutsopoulos H M, Gallagher JD, Decker *et al*. Herpes zoster in patients in SLE.*Arch.Pathol*.1971; 91:78-88.

62. Munther A. Khamashta, Silvia Pierangeli, E. Nigel Haris. Rheumatology, Fifth edition, *Volume* 2, Chapter 135, Antiphospholipid syndrome:Overview of pathogenesis, pg. 1351-59.

63. Murray and Nadel's Textbook of Respiratory Medicine, 4th Edition, *Vol*. 1, Part 2, Section F, Chapter 19, Micribiologic Diagnosis of Lower Respiratory Tract Infection, pg. 511- 530.

64. National Standard Method. Investigation of Bronchoalveolar Lavage, Sputum, and associated specimens. BS0757. Issued by Standards Unit.Department for Evaluations, Standards and Training Centre for Institutions.Issue no. 2.3, Issue date 11.12.2009.

65. Noel V, Lortholary O, Casassus P *et al*. Risk factors and prognostic influence of infection in a single cohort of 87 adults with systemic lupus erythermatosus. *Ann Rheum Dis* 2001; 60: 1141– 44.

66. Oh HM, Chng HH, Boey ML *et al*. Infections in systemic lupus erythematosus. *Singapore Med J* 1993; 34: 406–408.

67. Patel P, Werth V: Cutaneous lupus erythematosus: a review. *DermatolClin* 20: 373 – 385,v2002.

68. Paton NI, Cheong IK, Kong NC *et al*. Risk factors for infection in Malaysian patients with systemic lupus erythematosus. *Q J Med* 1996; 9: 531– 38.

69. Paul BJ, MuhammedFassaludeen, Nandakumar, Razia MV. Clinical profile of Systemic Lupus Erythematosus in Northern Kerala.*J Indian RheumatolAssoc* 2003; 11: 94-7.

70. Petri M, Genovese M. Incidence of and risk factors for hospitalizations in systemic lupus erythematosus: a prospective study of the Hopkins Lupus Cohort. *J Rheumatol* 1992; 19: 1559–1565.

71. Pickering M.C, Waiport M. J. et al, Boey M L *et al*. Links between complement abnormalities and SLE. *Rheumatology* 2000.Vol. 39.406-8.

72. Pryor BD, Bologna SG, Kahl LE: Risk factors for serious infection during treatment with cyclophosphamide and high-dose corticosteroids for systemic lupus erythematosus. *Arthritis Rheum* 1996, 39:1475-1482.

73. RenuSaigal, AmitKansal, Manoop Mittal , Yadvinder Singh, Hari Ram Maharia , Manish Juneja.Clinical profile of systemic lupus erythematosus patients at a tertiary care centre in Western India. *JIACM* 2011; 13(1): 27-32.

74. Reveille JD, Bartolucci A, Alarcon GS: Prognosis in systemic lupus erythematosus: negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. *Arthritis Rheum* 1990, 33:37–48.

75. Reyes CM, Navarra SV. Infections among Filipino patients with systemic lupus erythematosus.*Phil J Int Med* 2005; 43: 123–127.

76. RicardCervera, Munther A, Khamashta et al. Morbidity and Mortality in SLE during a five year period.*Medicine*, 1999.*Vol*.78. Pg. 167-75.

77. Rosner S, Ginzler EM, Diamond HS, *et al.*A multicenter study of outcome in SLE.II.Causes of death.*Arthritis Rheum* 1982; 25: 612-617.

78. Shyam C, Malaviya AN. Infection-related morbidity in systemic lupus erythematosus: a clinico-epidemiological study from northern India. .*RheumatolInt* 1996; 16: 1–3.

79. Staples PJ, Gerding DN, Decker JL, et al. Incidence of infection in systemic lupus erythematosus.*Arthritis Rheum*.1974; 17: 1-10.

80. Suh C.H, Jeong Y.S, Park H.C, *et al*. Risk factors for infection and role of CRP in Korean patients with SLE. *Clinical and experimental Rheumatology*.2001; 19: 191-4.

81. Tsao CH, Chen CY, Ou LS, Huang JL.Risk factors of mortality for Salmonella infection in systemic lupus erythematosus.*J Rheumatol* 2002; 29: 1214–18.

82. Tsokys GC, Moutsopoulos HM, Steinberg AD: Muscle involvement in systemic lupus erythematosus. *JAMA* 246: 766 – 768, 1981.

83. Urman JD, Lowenstein MB, Abeles M, *et al:* Oral mucosal ulceration in systemic lupus erythematosus. *Arthritis Rheum* 21: 58-61, 1978.

84. Urman JD, Rothfield NF: Corticosteroid treatment in systemic lupus erythematosus: survival studies. *JAMA* 1977, 238:2272–2276.

85. Vaidya S, Samant RS, Nadkar MY, Borges NE. SLE- review of two hundred and twenty patients. *JIRA* 1997; 5: 14-8.

86. Vyse TJ, Todd JA. Genetic analysis of autoimmune disease.*Cell* 1996; 85:311.

87. Wallace DJ, Podell T, Weiner J, *et al*.: Systemic lupus erythematosus—survival patterns: experience with 609 patients. *JAMA* 1981, 245:934–938.

88. Wei-I Tsai, Chien-Sheng Wu, Ping-Ning Hsu. Invasive fungal infections in systemic lupus erythematosus.10-year experience in a teaching hospital in Taiwan. Formosan *Journal of Rheumatology* 2009; 23:43-51.

89. Westley H. Reeves, Yi Li, HaoyangZhuang. Rheumatology, Fifth edition, *Volume* 2, Chapter 127, Autoantibodies in Systemic lupus erythematosus, pg. 1279-88.

90. Wysenbeek AJ, Leibovici L, Amit M, *et al*: Alopecia in systemic lupus erythematosus: Relation to disease manifestations. *J Rheumatol* 18:1185 – 1186, 1991.

91. Yi-Chan Tsai1, Chih-Lung Hou, Tsung-Chieh Yao, Li-Chen Chen, Tang-Her Jaing, Jing-Long Huang.Risk Factors and Bacterial Profiles of Urinary Tract Infections in Patients with Systemic Lupus Erythematosus.*Asian Pacific Journal Of Allergy And Immunology* (2007) 25: 155-161.

92. Yuhara T, Takemura H, Akama T *et al*. Predicting infection in hospitalized patients with SLE. *Internal Medicine* 1996; 35(8): 629-36.

Sl #	OP / IP #	Age	Sex	Episodes of Infection	Site of Infection	Pathogens	ESBL	MRSA	Immune Profile	C3 Levels	C4 Levels
1	82895	22	Female	One	Respiratory Tract	Pseudomonas aeruginosa			Anti-dsDNA	Decreased	
2	82111	52	Female	One	Urinary Tract	Escherichia coli	Yes				
3	93958	47	Female	One	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA		
					Blood	No Growth					
					CNS	No Growth					
4	105646	24	Male	One	Skin and Soft Tissue	Staphylococcus aureus		Yes	Anti-dsDNA	Decreased	
					Respiratory Tract	No Growth					
					Urinary Tract	No Growth					
5	110337	17	Female	One	Ear	Aspergillus flavus			Anti-dsDNA		
6	2317/06	26	Female	One	Urinary Tract	Escherichia coli			Anti-dsDNA	Decreased	
7	53603	30	Female	One	Respiratory Tract	Staphylococcus aureus		Yes	Anti-dsDNA		
8	48830	23	Female	One	Blood	Salmonella Typhi			Anti-dsDNA	Decreased	
					Urinary Tract	No Growth					
9	55319	24	Female		Urinary Tract	No Growth			Anti-dsDNA		
10	54541	47	Female		Respiratory Tract	No Growth					
11	52311	30	Female		Respiratory Tract	No Growth			Anti-dsDNA		
12	1787/11	28	Female	One	Urinary Tract	Escherichia coli			Anti-dsDNA	Decreased	
13	6177	35	Female	One	Urinary Tract	Pseudomonas aeruginosa	Yes		Anti-dsDNA	Decreased	Decreased
					Blood	Pseudomonas aeruginosa	Yes				
14	52045	32	Female		Respiratory Tract	No Growth			Anti-dsDNA	Decreased	Decreased
15	53703	24	Female	One	Respiratory Tract	Klebsiella pneumoniae	Yes				
16	51941	33	Female		Urinary Tract	No Growth			Anti-dsDNA		
17	53705	15	Female		Urinary Tract	No Growth					
18	7739	22	Female		Urinary Tract	No Growth			Anti-dsDNA		
19	8236	46	Female	One	Skin and Soft Tissue	Candida albicans			Anti-dsDNA	Decreased	Decreased
20	8117	30	Female		Urinary Tract	No Growth					
21	10134	41	Male	One	Respiratory Tract	Staphylococcus epidermidis			Anti-dsDNA		
22	9154	25	Female		Blood	No Growth					
					Urinary Tract	No Growth					
23	52223	26	Female		Urinary Tract	No Growth			Anti-dsDNA		
24	52234	34	Female		Respiratory Tract	No Growth			Anti-dsDNA	Decreased	Decreased
25	54921	18	Female	One	Blood	Klebsiella pneumoniae			Anti-dsDNA	Decreased	Decreased
					Urinary Tract	No Growth					
26	6031	15	Female		Respiratory Tract	No Growth			Anti-dsDNA	Decreased	
27	17494	21	Female	One	Urinary Tract	Citrobacter freundii	Yes				
					Blood	No Growth					
					GIT	No Growth					
28	11677	19	Female	One	Urinary Tract	Candida albicans			Anti-dsDNA		
29	46332	32	Female		Urinary Tract	No Growth					
30	55193	24	Female	One	Skin and Soft Tissue	Staphylococcus aureus		Yes			
31	19256	16	Female	One	Blood	Pseudomonas aeruginosa			Anti-dsDNA	Decreased	Decreased
32	26798	13	Male		Urinary Tract	No Growth					
33	26194	23	Female	Two	Respiratory Tract	Klebsiella pneumoniae	Yes		Anti-dsDNA	Decreased	Decreased
					Blood	Acinetobacter baumannii					
34	100504	19	Male	Two	Blood	No Growth					
					Respiratory Tract	Klebsiella pneumoniae					
					Urinary Tract	Escherichia coli	Yes				
35	29686	30	Female	One	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA		
36	31473	33	Female		GIT	No Growth					
37	1290/11	14	Female		Urinary Tract	No Growth			Anti-dsDNA		

38	32595	48	Female	One	Blood	No Growth				Decreased	
					Urinary Tract	Staphylococcus aureus		Yes			
39	46897	35	Female	One	Urinary Tract	Escherichia coli	Yes			Decreased	
40	38063	23	Female	Two	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA		
					Blood	Staphylococcus aureus					
41	36329	27	Female	One	Urinary Tract	Klebsiella oxytoca	Yes		Anti-dsDNA	Decreased	Decreased
42	1754/10	23	Female	Two	Urinary Tract	Staphylococcus epidermidis			Anti-dsDNA	Decreased	Decreased
					Urinary Tract	Escherichia coli	Yes				
43	1891/09	22	Female	One	Urinary Tract	Klebsiella pneumoniae			Anti-dsDNA	Decreased	Decreased
44	38642	24	Female	One	Blood	Staphylococcus aureus		Yes		Decreased	
					Urinary Tract	No Growth					
					Skin and Soft Tissue	No Growth					
45	40523	40	Female		GIT	No Growth			Anti-dsDNA		
46	41278	33	Male	One	Oral Cavity	Candida albicans				Decreased	
					Blood	No Growth					
47	42196	36	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
48	39432	16	Female		Urinary Tract	No Growth				Decreased	Decreased
49	41809	22	Female		Urinary Tract	No Growth			Anti-dsDNA		
					Blood	No Growth					
50	42274	25	Female	One	Skin and Soft Tissue	Pseudomonas aeruginosa	Yes			Decreased	Decreased
51	41789	19	Female	One	Urinary Tract	Acinetobacter baumannii			Anti-dsDNA		
					Blood	No Growth					
52	43418	30	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
53	43050	40	Female	Two	Blood	Klebsiella pneumoniae			Anti-dsDNA		
					Urinary Tract	Escherichia coli	Yes				
					CNS	No Growth					
54	68891	30	Female	One	Urinary Tract	Proteus mirabilis			Anti-dsDNA		Decreased
55	45305	30	Female	One	Respiratory Tract	Klebsiella pneumoniae	Yes			Decreased	Decreased
56	47569	32	Female		GIT	No Growth			Anti-dsDNA	Decreased	Decreased
57	45480	23	Female		GIT	No Growth					
					CNS	No Growth					
58	45732	27	Female	Three	Urinary Tract	Pseudomonas aeruginosa			Anti-dsDNA	Decreased	Decreased
					Urinary Tract	Staphylococcus aureus		Yes			
					Respiratory Tract	Klebsiella pneumoniae					
59	2301/09	16	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
60	47620	39	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
61	51296	20	Female	One	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA	Decreased	Decreased
62	52028	37	Female		Urinary Tract	No Growth				Decreased	Decreased
63	52583	15	Female	One	Urinary Tract	Pseudomonas aeruginosa			Anti-dsDNA	Decreased	Decreased
					Skin and Soft Tissue	Staphylococcus aureus					
64	51911	23	Female		Urinary Tract	No Growth			Anti-dsDNA		
65	53976	24	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
66	55212	16	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
67	53418	21	Female	One	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA	Decreased	Decreased
68	47302	24	Female		Urinary Tract	No Growth					
69	53926	32	Male		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
					Blood	No Growth					
70	56538	10	Male	One	Oral Cavity	Candida albicans				Decreased	Decreased
71	89217	21	Male		Blood	No Growth			Anti-dsDNA	Decreased	Decreased
					Urinary Tract	No Growth					
					Respiratory Tract	No Growth					

72	4679/12	22	Female	One	Urinary Tract	Candida tropicalis			Anti-dsDNA	Decreased	Decreased
73	96615	28	Female	One	Urinary Tract	Enterococcus faecalis			Anti-dsDNA	Decreased	Decreased
					Respiratory Tract	Klebsiella pneumoniae					
74	94300	21	Female	One	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA	Decreased	Decreased
					Skin and Soft Tissue	Pseudomonas aeruginosa	Yes				
75	71885	14	Female	One	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA	Decreased	Decreased
76	63511	19	Female	Two	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA	Decreased	Decreased
					Urinary Tract	Pseudomonas aeruginosa	Yes				
77	4048	15	Female	One	Urinary Tract	Escherichia coli				Decreased	Decreased
78	63167	12	Female	One	Urinary Tract	Enterococcus faecalis			Anti-dsDNA	Decreased	Decreased
79	65316	25	Female	One	Skin and Soft Tissue	Pseudomonas aeruginosa			Anti-dsDNA	Decreased	Decreased
80	68890	28	Female	One	Peritoneum	Pseudomonas aeruginosa	Yes		Anti-dsDNA	Decreased	Decreased
81	71028	21	Female	One	Respiratory Tract	Pseudomonas aeruginosa	Yes		Anti-dsDNA	Decreased	Decreased
82	75580	18	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
83	71883	17	Female	One	Urinary Tract	Escherichia coli			Anti-dsDNA	Decreased	Decreased
84	69794	20	Female	One	Blood	Staphylococcus epidermidis				Decreased	Decreased
					Urinary Tract	No Growth					
85	87361	22	Female	One	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA		
86	85300	25	Female	Two	Urinary Tract	Staphylococcus aureus		Yes	Anti-dsDNA	Decreased	Decreased
					Urinary Tract	Staphylococcus aureus		Yes			
87	260/08	37	Male		Urinary Tract	No Growth			Anti-dsDNA		
					Respiratory Tract	No Growth					
					Blood	No Growth					
88	49911	19	Male	One	Respiratory Tract	Klebsiella pneumoniae				Decreased	Decreased
89	26155	23	Male	One	Skin and Soft Tissue	Pseudomonas aeruginosa	Yes		Anti-dsDNA		
90	101164	21	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
91	100854	26	Male		Urinary Tract	No Growth				Decreased	Decreased
92	93118	18	Female	Two	Urinary Tract	Escherichia coli			Anti-dsDNA		
					Urinary Tract	Proteus mirabilis					
93	47565	25	Female	One	Ear	Pseudomonas aeruginosa	Yes		Anti-dsDNA		
94	89891	22	Female		Respiratory Tract	No Growth			Anti-dsDNA	Decreased	Decreased
95	1520/09	25	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
96	90785	18	Female		Urinary Tract	No Growth			Anti-dsDNA		
97	3720/05	30	Female	One	Urinary Tract	Klebsiella pneumoniae			Anti-dsDNA	Decreased	Decreased
98	108788	16	Female		Urinary Tract	No Growth				Decreased	Decreased
99	55390	13	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
100	88937	27	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
101	94736	19	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
102	2525/10	25	Female		Respiratory Tract	No Growth				Decreased	Decreased
103	54594	38	Female		Urinary Tract	No Growth				Decreased	Decreased
104	58903	26	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
105	109635	28	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
106	11315	28	Female	One	Urinary Tract	Escherichia coli	Yes		Anti-dsDNA	Decreased	Decreased
107	108611	24	Female		Blood	No Growth				Decreased	Decreased
					Urinary Tract	No Growth					
					Respiratory Tract	No Growth					
108	84898	22	Female		Urinary Tract	No Growth			Anti-dsDNA	Decreased	Decreased
109	8000	27	Female		Urinary Tract	No Growth				Decreased	Decreased
110	53976	24	Female	One	Respiratory Tract	Klebsiella pneumoniae	Yes			Decreased	